Ainouche, A., Bayer, R.J., Cubas, P. and Misset, M.-T. (2003). Phylogenetic relationships within tribe Genisteae (Papilionoideae) with special reference to genus *Ulex*. In: B.B. Klitgaard and A. Bruneau (editors). Advances in Legume Systematics part 10, Higher Level Systematics, pp. 239–252. Royal Botanic Gardens, Kew.

PHYLOGENETIC RELATIONSHIPS WITHIN TRIBE GENISTEAE (PAPILIONOIDEAE) WITH SPECIAL REFERENCE TO GENUS *ULEX*

ABDELKADER AINOUCHE*[1], RANDALL J. BAYER[2], PALOMA CUBAS[3], AND MARIE-THÉRÈSE MISSET[1]

[1]Laboratoire de Botanique, UMR CNRS Ecobio 6553, Université de Rennes-1, Rennes FR-35042 France
[2]CSIRO, Plant Ind., Molecular Systematics Lab, Australian National Herbarium, Canberra, Australia
[3]Departamento de Biologia Vegetal II, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain

Abstract

Molecular evidence presented here, from the literature and from this study, provide new insights into the systematics of the Genisteae *s.s.* Within this tribe, the evolutionary history of *Ulex* is investigated using phylogenetic analyses of two non-coding nuclear (ITS nrDNA) and plastid (*trn*L-*trn*F) sequences. *Ulex* represents a natural group, which is derived from within the *Genista-Cytisus* complex. A close relationship between *Ulex* and *Stauracanthus* is strongly supported by molecular data. *Ulex* appears to have evolved into two main lineages, which arose from a common diploid ancestor. One is represented by a single extant diploid species, *U. micranthus*, which is endemic in central Portugal. The second group includes all the remaining Euro-African diploid and polyploid taxa. The lack of resolution among the latter and their very weak molecular divergence are suggestive of a recent and rapid diversification of the gorses. The *trn*L-*trn*F sequence data also support *Lupinus* as a monophyletic group within Genisteae, that is distinct from the *Cytisus-Genista* complex.

Introduction

Within the Papilionoid legumes, the Genisteae (Adans.) Benth. *s.s.*, as defined by Polhill (1976) and re-arranged by Bisby (1981), represent a diverse tribe comprising about 20 genera and 450 species. The Genisteae are mostly woody shrubs which are essentially distributed in Europe and North Africa, and the Mediterranean region is viewed as their primary centre of diversification. Polhill distinguished the Genisteae from the other Genistoid tribes by a combination of morphological characters, primarily: stamen filaments joined into a closed tube with distinctly dimorphic anthers; leaves simple, unifoliolate or digitately three–many-foliolate; seeds exarillate, or if arillate only on a short side; calyx-lobes variously united, with a basically two-lipped calyx. During the two last decades, new evidence has been provided from biochemical investigations (Cristofolini and Feoli-Chiapella, 1977, 1984; Kinghorn

* author for correspondence: Kader.Ainouche@univ-rennes1.fr

and Balandrin, 1984; Van Wyk and Verdoorn, 1990; Wink, 1993), cladistic analyses of morphological and chemical data (Van Wyk and Schutte, 1995), and from recent molecular phylogenetic studies (Käss and Wink, 1997a; Crisp *et al.*, 2000; Kajita *et al.*, 2001), which allow a more precise circumscription of Polhill's concept of the Genisteae (Polhill, 1994). However, although much has been accomplished to improve our understanding of taxonomy and systematics within the Genisteae, delimitation of taxa and elucidation of their phylogenetic relationships at both the intergeneric and intrageneric levels remain complex and are still a matter of debate. Accordingly, the previously widely accepted intratribal arrangement of the Genisteae *s.s.* into two subtribes (Lupininae and Genistinae) and 20 genera (Bisby, 1981) has been variously re-considered by the authors, resulting in different phyletic interpretations and taxonomic treatments (Cristofolini and Feoli-Chiapella, 1984; Cristofolini, 1997; Talavera and Salgueiro, 1999a,b). For example, in their most recent proposal, Talavera and Salgueiro (1999a,b) recognised seven subtribes in the Genisteae; their re-arrangement involved changes of the limits, status and placement of several taxa at the generic, sectional and specific levels. In this context, we are especially interested in the genus *Ulex s.s.* (gorses).

Ulex s.s. is a small euploid series of thirteen to twenty perennial shrubby and spiny species and subspecies (Guinea and Webb, 1968; Cubas, 1999). Their natural distribution is geographically restricted to Western Europe and northwestern Africa, with the Iberian Peninsula regarded as their primary centre of diversity (Feoli-Chiapella and Cristofolini, 1981). Most taxa are very localised in these areas and only few are more widely distributed northward in Europe. The gorses are predominantly out-crossing, and exhibit different ploidy levels, diploids ($2n = 2x = 32$ chromosomes), tetraploids ($2n = 4x = 64$) and hexaploids ($2n = 6x = 96$) (see: Cubas, 1987; Misset and Gourret, 1996). The mode of formation of the polyploid taxa is still unknown, and no satisfactory phylogenetic hypothesis of relationships is available. Regardless of the number of species and subspecies described within *Ulex* all along its chequered taxonomic history, the most widely accepted infrageneric division was that of Rothmaler (1941). He defined two sections: Section *Neowilkommia*, with fasciculate spiny branches, corresponding to "Atlantic" and "subatlantic" taxa (such as, *U. minor* Roth, *U. gallii* Planch., *U. europaeus* L.); and Section *Sampaioa* comprising the "Mediterranean" and "submediterranean" gorses with non fasciculate branches (*U. argenteus* Welw. ex Webb, *U. parviflorus* Pourr., *U. micranthus* Lange, *U. densus* Welw. ex Webb). Such division found some support from morphological and serological data (Castro, 1945; Vicioso, 1962; Misset and Fontenelle, 1993). Moreover, *Ulex* has been variously circumscribed in the past (Fig. 1) to include other genera such as *Stauracanthus* (including *Nepa*) (Vicioso, 1962; Polhill, 1976) and *Echinospartum* (Rothmaler, 1941), and delimitation of these genera remains controversial (Bisby, 1981; Feoli-Chiapella and Cristofolini, 1981; Cubas, 1984). An explicit phylogenetic hypothesis for this genus is needed, not only to improve its systematics, but also to provide the historical framework for future comparative studies, in understanding the evolution of a variety of adaptive traits.

In this paper, we present (1) an analysis of the phylogenetic position of the genus *Ulex* within the Genisteae, and (2) a phylogenetic analysis of its intrageneric relationships, based on the recent molecular data available. As part of an ongoing project on the systematics and evolution of *Lupinus* and *Ulex* (Aïnouche and Bayer, 1999, 2000; Aïnouche *et al.*, in prep.), we present here the results obtained from cladistic analyses of two non coding DNA sequences: the internal transcribed spacers (ITS) of the nuclear rDNA repeats (Baldwin *et al.*, 1995); and the *trn*L-*trn*F chloroplast DNA region (Taberlet *et al.*, 1991), including the *trn*L intron and the *trn*L-*trn*F intergenic spacer (IGS).

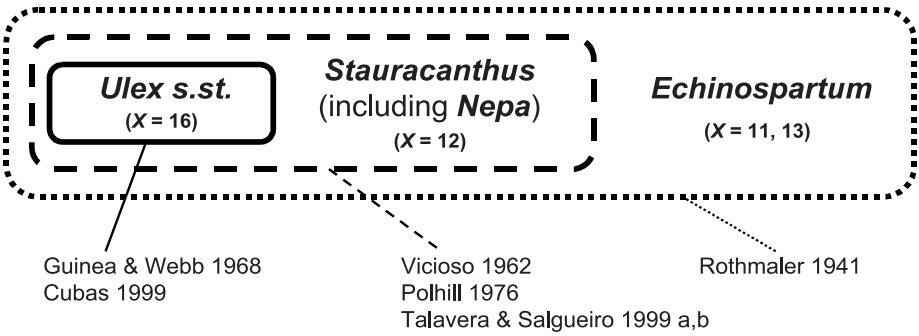


FIG. 1. Summary of difference *Ulex* concepts.

Materials and methods

Plant material

This study included different sets of samples, according to the inter- or intrageneric level of the analyses performed, and depending upon the availability of plant samples and sequences. Eleven *Ulex* samples are included in this study, for which both ITS nrDNA and *trn*L-*trn*F cpDNA (Intron and IGS) regions have been sequenced (see Table 1 for full information on taxa and their vouchers). They represent all species and almost all subspecies (11/13) recognised by Guinea and Webb (1968) in *Flora Europaea* in *Ulex s.s.* Two of the four subspecies of *U. parviflorus* are, however, missing from the analyses. Due to differences in taxonomic framework, these samples correspond to 11 of the 15 species described in *Flora Ibérica* (Cubas, 1999). As DNA sequencing of both nuclear and plastid DNA regions targeted in this study was not yet completed for some taxa, they are missing from the analyses. Despite this discrepancy, most of the groups of affinity circumscribed by Cubas (1984) are represented here. Considering our sampling, we have chosen, for more convenience, to follow the nomenclature of *Flora Europaea* in this preliminary analysis of the genus *Ulex*.

Twenty three additional taxa were included in the analyses in order to represent the tribe Genisteae, including 13 species of *Lupinus* (see Table 1), and three other taxa from tribes Crotalarieae (*Crotalaria podocarpa* D.C.) and Thermopsideae (*Thermopsis rhombifolia* (Nutt.) Richardson and *T. montana* Nutt.) were included as outgroups.

DNA isolation, PCR amplification, and Sequencing

The procedure followed is that previously described in Bayer and Starr (1998) and Aïnouche and Bayer (1999). Total genomic DNA was isolated from leaf tissue deriving either from herbarium specimens or from living plants. Double-stranded amplifications of ITS and *trn*L-*trn*F regions were performed for each DNA sample via the polymerase chain reaction in a volume of 100 µl, using: (1) the external ITS-1 and ITS-4 universal primers (White *et al.*, 1990) to amplify the complete ITS1 + 5.8S gene + ITS2 region; and (2) the external primers "c" and "f" (Taberlet *et al.*, 1991) for amplification of the *trn*L-*trn*F cpDNA region. The PCR amplification was performed for both targeted sequences via 30 cycles using 48° C for primer annealing. A 7 min final extension at 72° C followed cycle 30. Both strands of each of the PCR products were then directly sequenced via the dideoxy chain termination technique using the Big Dye Terminator cycle sequencing Ready Reaction Kit following the manufacturer's instructions. In addition to the external primers, universal internal primers were employed in the cycle sequencing reactions: the ITS-2 and the ITS-3

TABLE 1. List of samples included in this study.

Taxon	Geographic origin	Source[1]	GenBank sequence accession numbers: ITS nrDNA region ITS1 - ITS2	GenBank sequence accession numbers: *trn*L-*trn*F cpDNA region *trn*L intron / *trn*L-F spacer
Chamaecytisus mollis (Cav.) Greuter & Burdet	?	RBG Kew / 84327	GBANAF007472[2]	AF385414 / AF385940
Chamaespartium sagittale (L.) P.E. Gibbs	Bogève, Hte Savoie, France	AKA / CS 01		/ AF53871
Crotalaria podocarpa DC.	?	RBG Kew / 90928	GBANAF007469[2]	AF385412 / AF385938
Echinospartum boissieri (Spach) Rothm.	Jaén, Spain	MAF 148150		AF385415 / AF385941
Genista tinctoria L.	?	RBG Kew / 51334	GBANAF007471[2]	AF385413 / AF385939
Lupinus affinis J. Agardh	Oregon, USA	USDA 504315		/ AF538705
L. albus L.	Algeria, North Africa	INAE-DZ / M11		/ AF538702
L. angustifolius L.	Algeria, North Africa	AKA / M1		/ AF538699
L. argenteus Pursh	Washington, USA	USDA 504374		/ AF538706
L. cosentinii Guss.	Morroco, North Africa	INRAL-Fr / A 16		/ AF538697
L. hispanicus Boiss. & Reut.	Portugal	USDA 384555		/ AF538701
L. jaimehintoniana B.L. Turner	Oaxaca, Mexico F.D.	U of Texas – Herbarium / Hb Hinton G.B no. 26105		/ AF538704
L. luteus L.	Algeria, North Africa	AKA / M5		/ AF538700
L. mexicanus Cerv. ex. Lag.	Oaxaca, Mexico F.D.	USDA 14748		/ AF538703
L. micranthus Guss.	Algeria, North Africa	AKA / M8		/ AF538698
L. paraguariensis Chodat & Hassl.	S. Catarina, Brazil	CENARGEN 02828		/ AF538709
L. texensis Hook.	Texas, USA	USDA 577291		/ AF538707
L. villosus Willd.	Florida, USA	U of Florida - Herbarium D.Jones / FL 32608		/ AF538708
Pterospartum tridentatum (L.) Willk.	Pontevedra, Spain	LOU 24694		/ AF443654
Spartium junceum L.	Rennes, Brittany, France	Rennes (Campus)		/ AF538710
Stauracanthus boivinii (Webb) Samp.	Tlemcen, Algeria	HbUR / M 42	AF384338 / AF384339	AF385416 / AF385943
S. genistoides ssp. *genistoides* (Brot.) Samp.	Helva, Spain	MAF 7908	AF384340 / AF384341	AF385417 / AF385942
Thermopsis montana Nutt.	Montana, U.S.A.	HbUR / Ktm 101	AF 384336 / AF384337	AF385411 / AF385937
Ulex micranthus Lange	Portugal	HbUR / UM 62	AF384342 / AF384343	AF385418 / AF385944
U. minor Roth	Portugal	HbUR / 66 A	AF384344 / AF384345	AF385419 / AF385945
U. parviflorus ssp. *parviflorus* Pourr.	Languedoc, France	HbUR / UP 2	AF384346 / AF384347	AF385420 / AF385946
U. parviflorus ssp. *funkii* (Webb) Guinea	Oran, Algeria	HbUR / UP 40	AF384348 / AF384349	AF385421 / AF385947
U. argenteus ssp. *argenteus* Welw. ex Webb	Algarve, Portugal	HbUR / UAA 11	AF384350 / AF384351	AF385422 / AF385948

Table 1 continued.

U. argenteus ssp. *erinaceus* (Welw. ex Webb) Webb	Algarve, Portugal	HbUR / UAE 9	AF384352 / AF384353	AF385423 / AF385949
U. argenteus ssp. *subsericeus* (Cout.) Rothm.	Algarve, Portugal	HbUR / UAS 10	AF384354 / AF384355	AF385424 / AF385950
U. densus Welw. ex Webb	Extremadura, Portugal	HbUR / UD 7	AF384356 / AF384357	AF385425 / AF385951
U. europaeus ssp. *latebracteatus* (Mariz) Rothm.	Extremadura, Portugal	HbUR / UL 4	AF384358 / AF384359	AF385426 / AF385952
U. europaeus L. ssp. *europaeus*	Rennes, Brittany, France	Rennes (Campus)	AF384360 / AF384361	AF385427 / AF385953
U. gallii Planch.	Cap Frehel, Brittany, France	HbUR / UG 2	AF384362 / AF384363	AF385428 / AF385954

[1] HbUR = AKA: Abdelkader Aïnouche (first author); CENARGEN, EMBRAPA, Santa Catarina, Brazil; Herbier de l'Université de Rennes-1; INAE-DZ: Institut National Agronomique d'El Harrach, Algeria; INRAL: Institut National de Recherche Agronomique, Lusignan, France; RBG Kew: Royal Botanic Gardens, Kew, UK; MAF and LOU: Herbario Facultad Farmacia, Madrid, Spain; USDA: United States Department of Agriculture, ARS, WRPI Station, Pullman, Washington, USA. [2] Sequences from a previous work (Aïnouche & Bayer, 1999).

(from White *et al.*, 1990) to sequence separately the ITS1 and the ITS2 regions, respectively; and primers "d" and "e" (from Taberlet *et al.*, 1991) to sequence separately the *trn*L intron and the *trn*L- *trn*F IGS, respectively. The cycle sequencing products were analysed with an ABI 310 automated sequencer. No evidence of significant sequence heterogeneity was found in either the ITS or *trn*L- *trn*F regions among the polyploid taxa analysed here. The sequences have been deposited in GenBank Sequence Database (http://www.ncbi.nlm.nih.gov) and data matrices are available from the first author. All taxa included in this study are listed in Table 1, along with their geographic origin, the sample sources (vouchers), and DNA sequence accession numbers.

Sequence alignment and phylogenetic reconstruction

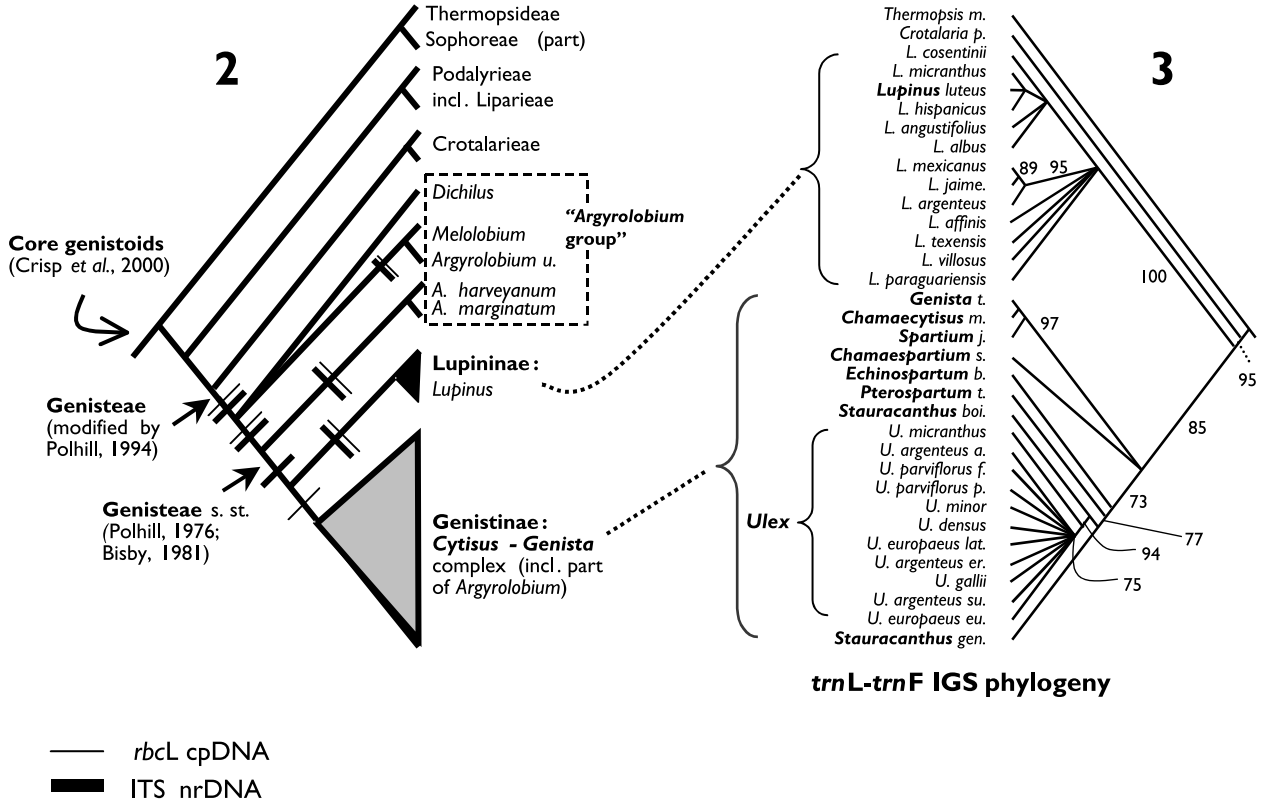
Sequences were verified manually and assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Only the non-coding sequences were taken into account. Three sequence data matrices were generated: (1) an IGS data matrix (34 taxa: 11 of *Ulex*, 13 of *Lupinus*, and 10 outgroups) to examine relationships of *Ulex* to outgroup taxa; (2) an ITS data matrix and (3) a *trn*L-*trn*F matrix comprised of 11 *Ulex* taxa and 6 outgroups for phylogenetic analysis of the genus. In the case of *Ulex*, separate data sets of the nuclear (biparentally inherited) and plastid (maternally inherited) sequences have been analysed separately prior to being pooled and analysed together following the "conditional combination" approach (Johnson and Soltis, 1998). Inspecting congruence between nuclear and chloroplast gene phylogenies is of particular interest in polyploid groups where reticulate evolution might have occurred (Johnson and Soltis, 1998; Seelanan *et al.*, 1997).

Multiple sequence alignment required inference of several insertion/deletion events (indels), especially in the *trn*L-*trn*F regions. The sequence data matrices were subjected to phylogenetic analyses using Fitch parsimony with PAUP (Swofford, 1998). *Thermopsis* has been used to root the trees in all analyses. Phylogenetic reconstructions were carried out via heuristic searches on unweighted characters and character states. The gaps have been excluded from the data matrices and unambiguous potentially informative indels have been coded and treated as additional multistate characters (with indels having the same position and length of one or more base pairs in the alignment scored as a single event). Bootstrap methods (using heuristic searches) was employed to examine the robustness of the various clades revealed in the trees. As incongruence was found among the separate gene (ITS and *trn*L-*trn*F) trees of *Ulex*, congruence between the two nuclear and plastid data sets was evaluated prior to combined analysis. Two statistical tests have been performed using PAUP: the character-based test for data set homogeneity (HT_F), and the significantly less parsimonious test (SLP_T) for character-state reconstruction on competing topologies (Johnson and Soltis, 1998).

Results and discussion

Present situation of tribe Genisteae *s.s.*

Since the last most comprehensive reviews of Polhill (1976) and Bisby (1981), the new evidence accumulated from serological investigations (Cristofolini and Feoli-Chiapella, 1984), and from cladistic analyses of either morphological, chemical or molecular data (Polhill, 1994; Van Wyk and Schutte, 1995; Käss and Wink, 1997a, 1997b; Aïnouche and Bayer, 1999; Crisp *et al.*, 2000; Kajita *et al.*, 2001), provide a more accurate picture of the tribe Genisteae *s.s.* Figure 2 presents a diagram summarising the present circumscription of the Genisteae in the context of the "core genistoid" (defined by Crisp *et al.*, 2000), as supported by recent molecular phylogenetic analyses. As can be seen from this diagram, both *rbc*L and ITS sequence data support a common



FIGS. 2–3. Phylogenetic position of *Ulex* and *Lupinus* within the tribe Genisteae. **Fig 2.** Phylogenetic position and circumscription of the Genisteae *sensu* Polhill (1976, 1994) redrawn from recent ITS and *rbc*L data (after Käss and Wink, 1997a; Aïnouche and Bayer, 1999; Crisp *et al.*, 2000; Kajita *et al.*, 2001). **Fig. 3.** Phylogenetic position of *Ulex* and *Lupinus*. A *trn*L-*trn*F – IGS based phylogeny of 34 genistoid taxa is presented (heuristic search; strict consensus of 1895 maximum parsimonious trees of 169 steps length; CI = 0.793; RI = 0.922). Bootstrap values (from 100 replicates) are indicated on the branches.

ancestor for the genera grouped by Polhill (1976, 1981) and Bisby (1981) in the Genisteae *s.s.*, including *Lupinus* and part of *Argyrolobium*. These data also demonstrate that all the exemplars from *Argyrolobium, Melolobium* and *Dichilus* (usually classified in the tribe Crotalarieae), included in different phylogenetic analyses of the legumes, are closely related to the Genisteae with which they form a well supported clade that is sister group to the remainder of the Crotalarieae (Käss and Wink, 1997a; Crisp *et al.*, 2000; Kajita *et al.*, 2001). Therefore, although the sampling of these genera is still weak, the available molecular phylogenetic evidence agrees well with the recent exclusion of the *Argyrolobium* group (including the mainly south African *Argyrolobium, Melolobium, Dichilus*, and the south American *Sellocharis* and *Anartrophyllum*) from the Crotalarieae and their transfer to the Genisteae, based on morphology and chemistry (Polhill, 1994; Van Wyk and Schutte, 1995; Nysschen *et al.*, 1998). Accordingly, the new concept of the tribe include the genistoid taxa with basically two-lipped calyx and a trifid lower calyx lip, and which contain quinolizidine alkaloids of a-pyridone type (characterising the Genisteae). Albeit the weak divergence found at the base of the tribe, and except some *Argyrolobium* species, the members of the *Argyrolobium* group appear to have early diverged from the common ancestor of the Genisteae (see Fig. 2; Käss and Wink, 1997a; Crisp *et al.*, 2000; Kajita *et al.*, 2001). The molecular data also reveal the polyphyletic nature of the genus *Argyrolobium*, whose position was always controversial (Polhill, 1976, 1981; Van Wyk and Schutte, 1995). Käss and Wink (1997a) considered that only the Mediterranean species (e.g. *A. zanonii* (Turra) P. W. Ball) are related to the *Cytisus-Genista* complex, while the south African taxa would be genetically closer to the Crotalarieae. In their treatment of the Leguminosae, Talavera and Salgueiro (1999a) included *Argyrolobium* in the Thermopsideae. Although it is obvious that this group is artificial and needs to be carefully analysed and reorganised, the molecular data summarised here from the literature suggest, however, that it belongs to the Genisteae. Circumscription and elucidation of the *A. arveyanum-A. marginatum* group are of great interest to best understand the evolutionary history of the Genisteae *sensu* Bisby (1981). Within the latter, the *Cytisus-Genista* complex (including *Ulex*) and *Lupinus* are each supported as monophyletic groups (Fig. 2). The molecular data suggests that lupines form a distinct natural group that has diverged independently from the ancestor of the *Cytisus-Genista* complex (Käss and Wink, 1997b; Aïnouche and Bayer, 1999). Based upon the available ITS and *rbc*L data, phylogenetic relationships among genera were only poorly resolved at the base and within the latter complex, as demonstrated by short inter-nodes and weak bootstrap support (Käss and Wink, 1997a; Kajita *et al.*, 2001). However, there are clues from these studies and from a very recent molecular analysis of *Cytisus* and its related genera (Cubas *et al.*, 2002) which lend support to the distinction between the *Cytisus*-group (including: *Argyrocytisus*, *Calicotome*, *Chamaecytisus*, *Cytisophyllum*, *Cytisus* and *Spartocytisus*) and the *Genista*-group. *Ulex* was always placed outside of the *Cytisus*-group and close to the members of the *Genista*-group.

As part of an ongoing study of the *trn*L-*trn*F phylogeny of the Genisteae, started in our laboratories, a preliminary phylogenetic analysis of a *trn*L-*trn*F IGS data set already available is presented here (Fig. 3). This data set includes exemplars from 34 taxa which represent 11 genera belonging to: Thermopsideae (1), Crotalarieae (1), and Genisteae (9) (Table 1). Among the latter, *Ulex*, which is the focus of this paper, is represented by 11 taxa. Thirteen *Lupinus* species, originating from the main centres of diversity of the genus in the Old and the New World, are included in this data set to represent the largest (~200/473) and the only genus in the tribe Genisteae that has an amphiatlantic distribution. Although the sampling is still limited, the preliminary *trn*L-*trn*F IGS results are in general agreement with the phylogenetic pattern redrawn from the ITS and *rbc*L data (Fig. 2). All representatives of the *Cytisus-Genista* complex (including *Ulex*) share a common ancestor (bootstrap = 85%) and are isolated from the well supported *Lupinus* clade (bootstrap = 100%). Thus, the IGS data lend additional support to a higher level taxonomic treatment of the genus *Lupinus* within the Genisteae. Both Bisby (1981) and

Talavera and Salgueiro (1999a,b) restored the subtribe *Lupininae*. However, as well as ITS and *rbc*L data (cited elsewhere), the *trn*L-*trn*F IGS provide any evidence supporting the proposition of Talavera and Salgueiro (1999a,b) to divide the *Cytisus-Genista* complex into six distinct subtribes. Within this complex, *Ulex* forms a well supported clade whose sister relationship to *Stauracanthus* is well resolved (bootstrap = 94%) in the IGS phylogeny (will be discussed later). While the *trn*L-*trn*F IGS sequences appear to be promising for high level phylogenetic inference in the Genisteae, they provide only a few informative characters at the intrageneric level as can be seen in *Ulex* and *Lupinus* (Fig. 3). However, these additional characters can supplement other sequence data sets to increase phylogenetic resolution.

Phylogenetic relationships within *Ulex*

The results of an ongoing molecular systematic study of *Ulex* and allied genera (Aïnouche *et al.*, in prep.) are summarised below.

Potentially informative characters were more numerous in the ITS region than in *trn*L-*trn*F (54 *vs.* 37 substitutions, and 6 *vs.* 14 coded indels, respectively). Each of the ITS and *trn*L-*trn*F sequence data sets have been analysed separately, and yielded phylogenetic trees with high consistency indices: 0.956 and 0.947, for ITS and *trn*L-*trn*F trees, respectively (results not shown). Inspection and comparison of the topologies resulting from these separate analyses (involving the same taxa) revealed a similar pattern of relationships at the intergeneric level, with only two differences: one concerned the support to the *Genista-Chamaecytisus* sister relationship (much higher in the plastid tree than in the ITS one); and the other concerned the relative position of the two species of *Stauracanthus* (to each other), which were resolved in the ITS tree but not in the *trn*L-*trn*F phylogeny. A few other differences between the data sets concerned the position of *U. micranthus*, which was resolved in the ITS tree but not in the the *trn*L-*trn*F phylogeny, and some subclades that were moderately to well supported by either ITS or *trn*L-*trn*F sequences (will be discussed below). The partition homogeneity test (HT_F) performed on the overall nuclear and plastid data indicated an insignificant level of incongruence between the two data sets (P value = 0.83 > 0.05). Additionally, the use of the significantly less parsimonious test (SLP_T) showed a significant proportion of conflicting characters between the two data sets (P values = 0.059 and 0.32). Thus, phylogenetic analyses were conducted using all the data available pooled into a single matrix, according to the conditional combination approach.

A strict consensus tree was reconstructed from the 520 maximum parsimonious trees (430 steps length; CI= 0.881; RI= 0.778) generated by a heuristic search. In its overall topology, the combined phylogeny (presented in Fig. 4) reflects the ITS topology (not shown) and includes a subclade (grouping *U. europaeus* subsp. *latebracteatus* and *U. gallii*),which is poorly supported in the *trn*L-*trn*F phylogeny (not shown). This tree demonstrates the close relationship between *Stauracanthus* and *Ulex*, which fall together in a strongly supported clade (bootstrap 100%). *Stauracanthus* appears as a paraphyletic grade to *Ulex*. This close relationship between the two taxa was not altered, even when other Genisteae, such as *Echinospartum boissieri* (Spach) Rothm. and *Chamaespartium tridentatum* (L.) P.E. Gibbs, supposedly close to *Ulex*, were introduced in separate ITS or *trn*L-*trn*F analyses. Furthermore, this is also illustrated in the *trn*L-*trn*F IGS phylogeny shown in Fig. 3. These results are congruent with the previous delimitation of *Ulex* based on morphology and serology (Vicioso, 1962; Polhill, 1976; Feoli-Chiapella and Cristofolini, 1981). However, in most of the recent taxonomic revisions, *Stauracanthus* and *Ulex s.s.* were maintained as separate genera based on some morphological criteria, chromosome numbers ($x = 12$ and $x = 16$, respectively), and their ecologically disjunct distributions (Guinea and Webb, 1968; Cubas, 1999; Paiva and Coutinho, 1999; Talavera and Salgueiro, 1999a,b). Talavera and Salgueiro, 1999a,b) grouped *Stauracanthus* and *Ulex* in a new subtribe, *Ulicinae*, within the tribe Genisteae. Based on the molecular phylogenetic results presented here, it seems more appropriate to include *Stauracanthus* in *Ulex* rather than to group them at a higher level. The inclusion of *Echinospartum,*

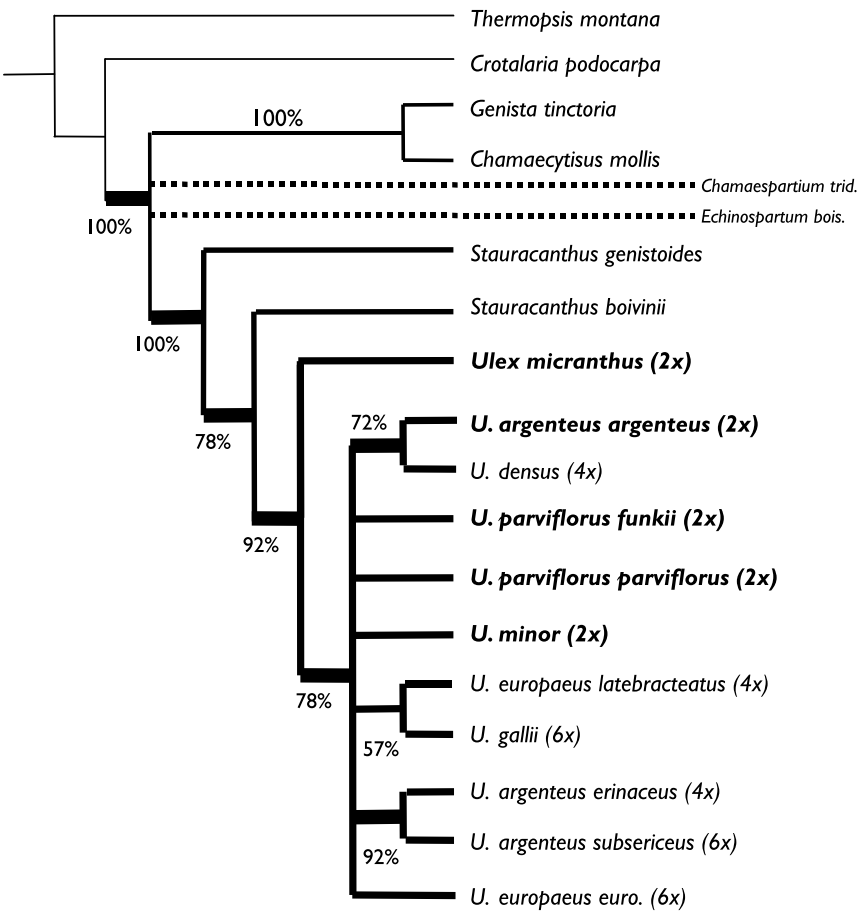


FIG. 4. Phylogeny of *Ulex* and outgroup taxa based on the combined ITS and *trn*L-*trn*F sequence data. Presented is a strict consensus reconstructed from 520 maximum parsimonious trees (430 steps length; CI = 0.881; RI = 0.778) generated by heuristic search. Chromosome numbers follow taxon names. Diploid taxa are highlighted in bold type. Bootstrap values (1000 replicates) are indicated on the branches. Dotted lines mapped in the cladogram indicate the relative position held by *Chamaespartium tridentatum* and *Echinospartum boissieri* when these taxa were each introduced into separate analyses of the ITS or the *trn*L-*trn*F data sets, respectively.

together with *Ulex* and *Stauracanthus,* in a broad *Ulex* group found no support from our *trn*L-*trn*F data set. Instead, the controversial *Pterospartum tridentatum* (syn. to *Chamaespartium tridentatum*) appears as the closest taxon to the *Stauracanthus-Ulex* group (bootstrap support = 77% in Fig. 3), based on the molecular data presently available (from this study; Käss and Wink, 1997a; Cubas *et al.*, 2002).

Ulex s.s. is clearly monophyletic, and appears to have evolved through two sister lineages. One is presently represented by one extant species *U. micranthus*, a diploid (2*x*) species endemic to central Portugal, and the other lineage contains the four remaining diploid taxa and all the polyploid (4*x* and 6*x*) ones (hereafter designated as the "polyploid clade"), regardless of their Atlantic or Mediterranean origin. This general topology remains unchanged when the polyploid *Ulex* taxa are excluded from the analysis. Thus, the present results support an origin of *Ulex* from a common diploid ancestor (with $x = 16$), but do not provide any evidence supporting either the taxonomic subdivision of the genus in two sections, *Neowilkommia* and *Sampaioa* (Rothmaler, 1941) or the early *Ulex* phylogenetic hypothesis of Castro (1945).

Within the largely unresolved "polyploid clade" (which is sister to *U. micranthus*), some diploid-polyploid relationships, however, do emerge, and there are some clues which make questionable the taxonomic treatment of species such as *U. argenteus* and *U. europaeus* (Aïnouche *et al.*, in prep.). The three subspecific samples of the morphologically and geographically homogeneous species *U. argenteus* do not form a monophyletic group. They are placed in different subclades, which are supported by ITS characters: the tetraploid subsp. *erinaceus* is strongly related to the hexaploid subsp. *subsericeous* (bootstrap = 92%), while the diploid subsp. *argenteus* is closely related to the morphologically and geographically distinct *U. densus* (tetraploid), with which it shares identical ITS sequences. Also *U. europaeus* was not monophyletic in either the ITS, the *trn*L-*trn*F or in the combined phylogeny, but subsp. *latebracteatus* (tetraploid) was related to *U. gallii* (hexaploid), based on the maternally inherited plastid characters. However, this relationship was only poorly supported (bootstrap = 57%).

These results are suggestive of some divergent and reticulate relationships within the *Ulex* "polyploid clade". However, there were not enough phylogenetically informative characters in the ITS and *trn*L-*trn*F sequences supporting the nodes (1 or 2 character changes) to allow reliable inference of species relationships. Based on a preliminary sampling analysed here, these results represent interesting clues which should help initiate further, more accurate investigations. The low level of ITS and *trn*L-*trn*F sequence divergence is suggestive of a recent and rapid diversification of the gorses, that involved polyploidisation as a major mechanism of speciation. Thus, further investigations (including, a wide sampling of the species/population diversity of *Ulex,* and other complementary and appropriate genetic approaches should be conducted to improve the phylogenetic resolution in this genus, particularly concerning the diploid-polyploid relationships. This is of particular interest for the ubiquitous hexaploid European gorse, *Ulex europaeus* subsp. *europaeus*, which has rapidly extended its original range from the Iberian Peninsula northwards in Europe, and is now an invasive weed in Oregon, California, Hawai, La Reunion, Australia and in New Zealand, following introductions during the last two centuries.

Conclusion

Recent molecular data presented here from our own studies and available from the literature, provide a more accurate picture of the tribe Genisteae *sensu* Polhill. Within this tribe, the genus *Ulex* represents a monophyletic group whose position is best established. *Lupinus* is clearly distinct from all the other Genisteae, while *Ulex* appears as one of the more derived extant genera within the *Genista-Cytisus* complex, with *Stauracanthus* as its closest relative. *Ulex* initially evolved as two lineages which recently arose from a common diploid ancestor. Although the molecular data

presented above provide new insights into the systematics and evolution of gorses, further progress to improve our understanding of their evolutionary history needs additional studies in order to elucidate a number of unresolved points. Among the latter having priority in our ongoing investigations, the questions to be addressed include the clarification of the diploid-polyploid relationships within *Ulex*.

Acknowledgements

These studies were supported by the research unit UMR-CNRS 6553 Ecobio - University of Rennes (France) and by the CSIRO - Plant Industry - Australian National Herbarium of Canberra (Australia). They are gratefully acknowledged.

Literature cited

Aïnouche, A.K. and Bayer, R.J. (1999). Phylogenetic relationships in *Lupinus* (Fabaceae: Papilionoideae) based on internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA. *American Journal of Botany* 86: 590–607.

Aïnouche, A.K. and Bayer, R.J. (2000). Genetic evidence supports the new anatolian lupine accession, *Lupinus anatolicus*, as an Old World "rough-seeded" lupine (Section *Scabrispermae*) related to *L. pilosus*. *Folia Geobotanica et Phytotaxonomica* 35: 83–95.

Aïnouche, A.K., Misset, M.T. and Bayer, R.J. (in prep.). Molecular systematics and evolution of *Ulex s.l.* (Fabaceae) inferred from non coding nuclear (ITS nrDNA) and plastid (*trn*L-*trn*F) sequence data. *Plant Systematics and Evolution*.

Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. and Donoghue, M.J. (1995). The ITS region of nuclear ribosomal DNA: A valuable source of evidence on Angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

Bayer, R.J. and Starr, J.R. (1998). Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trn*L intron and the *trn*L/*trn*F intergenic spacer. *Annals of the Missouri Botanical Garden* 85: 242–256.

Bisby F.A. (1981). Genisteae (Adanson) Benth. In: R.M. Polhill and P.H. Raven (Editors). Advances in legume systematics, part 1, pp. 409–425. Royal Botanic Gardens, Kew.

Castro de, D. (1945). Alguns dados cariologicos para a systematica dos generos *Echinospartum* (Spach) Rothm., *Stauracanthus* Link, *Nepa* Webb e *Ulex* L. *Boletim da Sociedade Broteriana* 2nd *ser.*: 525–539.

Crisp, M.D., Gilmore, S. and Van Wyk, B-E. (2000). Molecular phylogeny of the Genistoid tribes of Papilionoid legumes. In: P.S. Herendeen and A. Bruneau (editors). Advances in legume systematics, part 9, pp. 249–276. Royal Botanic Gardens, Kew.

Cristofolini, G. (1989). A serological contribution to the systematics of the genus *Lupinus* (Fabaceae). *Plant Systematics and Evolution* 166: 265–278.

Cristofolini, G. (1997). The biodiversity of the Leguminosae-Genisteae and its genera. *Lagascalia* 19: 121–128.

Cristofolini, G. and Feoli-Chiapella, L. (1977). Serological systematics of the tribe Genisteae (Fabaceae). *Taxon* 26: 43–56.

Cristofolini, G. and Feoli-Chiapella, L. (1984). Origin and diversification of Genisteae (Fabaceae): a serosystematic purview. *Webbia* 38: 105–122.

Cubas, P. (1984). Estudio taxonomico de los generos *Ulex* L. y *Stauracanthus* Link en la peninsula iberica. Thesis doctoral 211/84, Universidad Complutense de Madrid, Spain.

Cubas, P. (1987). Numeros cromosomaticos en *Ulex* L. y *Stauracanthus* Link (Genisteae, Papilionaceae). *Anales del Jardin Botánico de Madrid* 43: 217–233.

Cubas, P. (1999). *Ulex* L. In: S. Talavera, C. Aedo, S. Castroviejo, C. Romero Zarco, L. Sáez, F.J. Salgueiro and M. Velayos (editors). Flora Iberica Plantas vasculares de la península Iberica e Islas Baleares. Vol. 7(1) Leguminosae, pp. 212–239. Real Jardin Botanico, CSIC, Madrid.

Cubas, P., Pardo, C. and Tahiri, H. (2002). Molecular approach to the phylogeny and systematics of *Cytisus* (Leguminosae) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (*trn*L-*trn*F intergenic spacer). *Plant Systematics and Evolution* 233: 223–242.

Feoli-Chiapella, L. and Cristofolini, G. (1981). Serological contributions to the systematics of *Ulex* (Genisteae-Fabaceae) and allied genera. *Nordic Journal of Botany* 1: 723–729.

Guinea, E. and Webb, D.A. (1968). *Ulex* L. In: T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters and D.A. Webb (editors). Flora Europaea 2, p. 102. Cambridge University Press, Cambridge.

Johnson, L.A. and Soltis, D.E. (1998). Assessing congruence: empirical examples from molecular data. In: D.E. Soltis, P.S. Soltis and J.J. Doyle (editors). Molecular systematics of plants, pp. 297–347. Kluwer Academic Publishers, Boston.

Kajita, T., Ohashi, H., Tateishi, Y., Bailey, C.D. and Doyle, J.J. (2001). RbcL and legume phylogeny with particular reference to *Phaseolus*, Milettiae and allies. *Systematic Botany* 26: 513–536.

Käss, E. and Wink, M. (1997a). Phylogenetic relationships in the Papilionoideae (Family Leguminosae) based on nucleotide sequences of cpDNA (ITS 1 and ITS 2). *Molecular Phylogenetics and Evolution* 8: 65–88.

Käss, E. and Wink, M. (1997b). Molecular phylogeny and phylogeography of *Lupinus* (Leguminosae) inferred from nucleotide sequences of the *rbcL* gene and ITS 1 + 2 regions of rDNA. *Plant Systematics and Evolution* 8: 139–167.

Kinghorn, A.D. and Balandrin, M.F. (1984). Quinolizidine alkaloids of Leguminosae: structural, types, analysis, chemotaxonomy and biological activities. In: S.W. Pelletier (editor). Alkaloids: chemical and biological perspectives, pp. 105–148. John Wiley and Sons, New York.

Misset, M.T. and Fontenelle, C. (1993). Antigenic relations among species of the genus *Ulex* L. (Faboideae-Genisteae) determined by cross immuno-electrophoresis and immunoblotting. *Evolutionary Trends in Plants* 7: 23–28.

Misset, M.T. and Gourret, J.P. (1996). Flow cytometric analysis of the different ploidy levels observed in the genus *Ulex* L. (Faboideae-Genisteae) in Brittany (France). *Botanica Acta* 109: 72–79.

Nysschen, de, A.M., Van Wyk, B.E., and Van Heerden, F.R. (1998). Seed flavonoids of the Podalyriae and Liparieae (Fabaceae). *Plant Systematics and Evolution* 212: 1–11.

Paiva, J. and Coutinho, A.X.P. (1999). *Stauracanthus* Link. In: S. Talavera, C. Aedo, S. Castroviejo, C. Romero Zarco, L. Sáez, F.J. Salgueiro and M. Velayos (editors). Flora Iberica Plantas vasculares de la península Iberica e Islas Baleares. Vol. 7(1) Leguminosae, pp. 240–245. Real Jardin Botanico, CSIC, Madrid.

Polhill R.M. (1976). Genisteae (Adans.) Benth. and related tribes (Leguminosae). *Botanical Systematics* 1: 143–368.

Polhill, R.M. (1981). Tribe 29. Crotalarieae (Benth.) Hutch. In: R.M. Polhill and P.H. Raven (editors). Advances in Legume Systematics, part 1, pp. 191–208. Royal Botanic Gardens, Kew.

Polhill, R.M. (1994). Classification of the Leguminosae. In: F. Bisby, J. Buckingham and J.B. Harborne (editors). Phytochemical Dictionary of the Leguminosae, pp. XXV – XIVII. Chapman and Hall, London.

Rothmaler, W. (1941). Revision der Genisteen. 1 Monographien der Gattungen um Ulex. *Botanische Jahrbücher für Systematik* 72: 69–116.

Seelanan, T., Schnabel, A. and Wendel, J.F. (1997) Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany* 22: 259–290.

Swofford, D.L. (1998). PAUP 4.0: Phylogenetic analysis using parsimony. Beta version 4.0b4a. Sinauer Associates, inc., Illinois, USA.

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Talavera, S. and Salgueiro, F.J. (1999a). Sobre el tratamiento de la familia Leguminosae en Flora Ibérica. *Lagascalia* 21: 155–222.

Talavera, S. and Salgueiro, F.J. (1999b) Sobre el tratamiento de la tribu Cytiseae Bercht. & J. Presl (Papilionoideae, Leguminosae) en Flora Ibérica. Anales Jardin Botanico de Madrid 57: 200–218.

Van Wyk, B-E. and Verdoorn, G.H. (1990). Alkaloids as taxonomic characters in the tribe Crotalarieae (Fabaceae). *Biochemical Systematics and Ecology* 18: 503–515.

Van Wyk, B-E. and Schutte, A.L. (1995). Phylogenetic relationships in the tribes Podalyrieae, Liparieae and Crotalarieae. In: M. Crisp and J.J. Doyle (editors). Advances in Legume Systematics, part 7, Phylogeny, pp. 283–308. Royal Botanic Gardens, Kew.

Vicioso, C. (1962). Revision del genero *Ulex* en Espana. *Bolétin del Instituto Forestal de Investigaciónes y Experiencias (Madrid)* 80: 1–59.

White, T.J., Bruns, Y., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal RNA genes for phylogenetics. In: M. Innis, D. Gelfand, J. Sninsky. and T. White (editors). PCR protocols: a guide to methods and applications, pp; 315–322. Academic Press, San Diego.

Wink, M. (1993). Quinolizidine alkaloids. In: P.G. Waterman, Methods in Plant Biochemistry, Alkaloids and sulphur compounds, Vol. 8, pp. 197–239. Academic Press, London and New York.