

Molecular phylogenetic evidence for the geographic origin and classification of Canary Island *Lotus* (Fabaceae: Loteae)

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Abstract

Molecular phylogenetic analyses of Macaronesian *Lotus* and related genera were conducted to assess their biogeographic history and taxonomy. Macaronesian *Lotus*, which are typically classified within one of two subgenera, *Lotus* subgenus *Pedrosia* or *L.* subg. *Rhyncholotus*, are diagnosed by the presence of a forked or toothed style and differences in corolla morphology. Maximum parsimony and Bayesian analyses of internal transcribed spacer sequences identify a well-supported northwest African-Cape Verde Island clade that includes all members of *Lotus* subgenus *Pedrosia* + *L.* subg. *Rhyncholotus*. There is modest support for two independently nested clades containing the Canary Island species and two non-Canarian species, *Lotus assakensis* from Africa and *Lotus azoricus* endemic to the Azores. Biogeographic reconstruction based on a parsimony topology unequivocally identifies an African origin for the Canary Island group with subsequent back dispersal to the African continent and a single dispersal event to the Azores. A phylogeographic assessment of colonization and diversification patterns suggests that geographic isolation via interisland colonization of ecologically similar habitats is the primary mode of species diversification in Canary Island *Lotus*.

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1. Introduction

The Canary Island flora has been the subject of numerous floristic-based studies ranging from the composition and distribution of the flora (Bramwell, 1972a; Hansen and Sunding, 1993; Santos-Guerra, 1999) to its biogeography and ecology (Bramwell and Bramwell, 1976). With the application of molecular-based methods, however, investigators have been able to address in greater detail questions about its geographic origins and ecological diversification. These molecular-based studies have employed data from allozymes (see review in

Francisco-Ortega et al., 2000) restriction fragment length polymorphisms (Barber et al., 2000; Francisco-Ortega et al., 1995) and nucleotide sequences from chloroplast DNA, or the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (see review in Baldwin et al., 1998). These studies have largely focused on the geographic origins of the Canary Island flora and its diversification within an insular environment.

In addition to the biogeographic and ecological insight provided by molecular phylogenetic studies, independent assessments of the taxonomy of insular endemic plant groups have also been conducted. Among the more notable plant genera that have been the focus of taxonomic reevaluation include *Crambe* (Brassicaceae) (Francisco-Ortega et al., 1997a), three endemic genera of

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the Asteraceae (Francisco-Ortega et al., 1997a, 2001); the genus *Aeonium* (Crassulaceae; Mort et al., 2001), endemic species of *Sideritis* [(Lamiaceae) Barber et al., 2000, 2002] and island broom genera of the Genisteae (*Adenocarpus*, *Genista*, and *Teline*; Percy and Cronk, 2002). In this study, we examine the biogeography and taxonomy of Macaronesian-endemic species of *Lotus* L., a temperate-herbaceous genus of legumes with a large center of diversity in the Mediterranean region.

The Macaronesian Islands comprise five Atlantic Ocean archipelagos, which include the Azores, Madeira, Selvagens, the Cape Verde Islands and the Canary Islands (Fig. 1). The Canarian archipelago includes seven islands (El Hierro, Fuerteventura, Gran Canaria, La Gomera, Lanzarote, La Palma and Tenerife) located approximately 100 km off the western coasts of Europe and Africa and situated between 27° and 30° N latitude. The islands are volcanic in origin and range in age from

approximately 21 million years (Myr) to 0.8 Myr (Carracedo, 1994). A wide range of topological and climatological diversity also characterizes the Canaries and has produced vegetation zones ranging from semi-desert succulent scrub (0–700 m) to pine forests (1200–2000 m) and montane scrub (1900–2500 m).

1.1. The genus *Lotus*

Lotus is a large (150 spp.), cosmopolitan genus that occupies two major centers of diversity, the Mediterranean region (including portions of Europe, Africa, and western Asia) and western North America. It is one of about 10 genera within the tribe Loteae (Polhill, 1981; Sokoloff, 1998) and is the only genus in the tribe with an intercontinental distribution. Molecular phylogenetic analyses indicate that tribe Loteae is monophyletic and that *Lotus* comprises two distinct clades, which

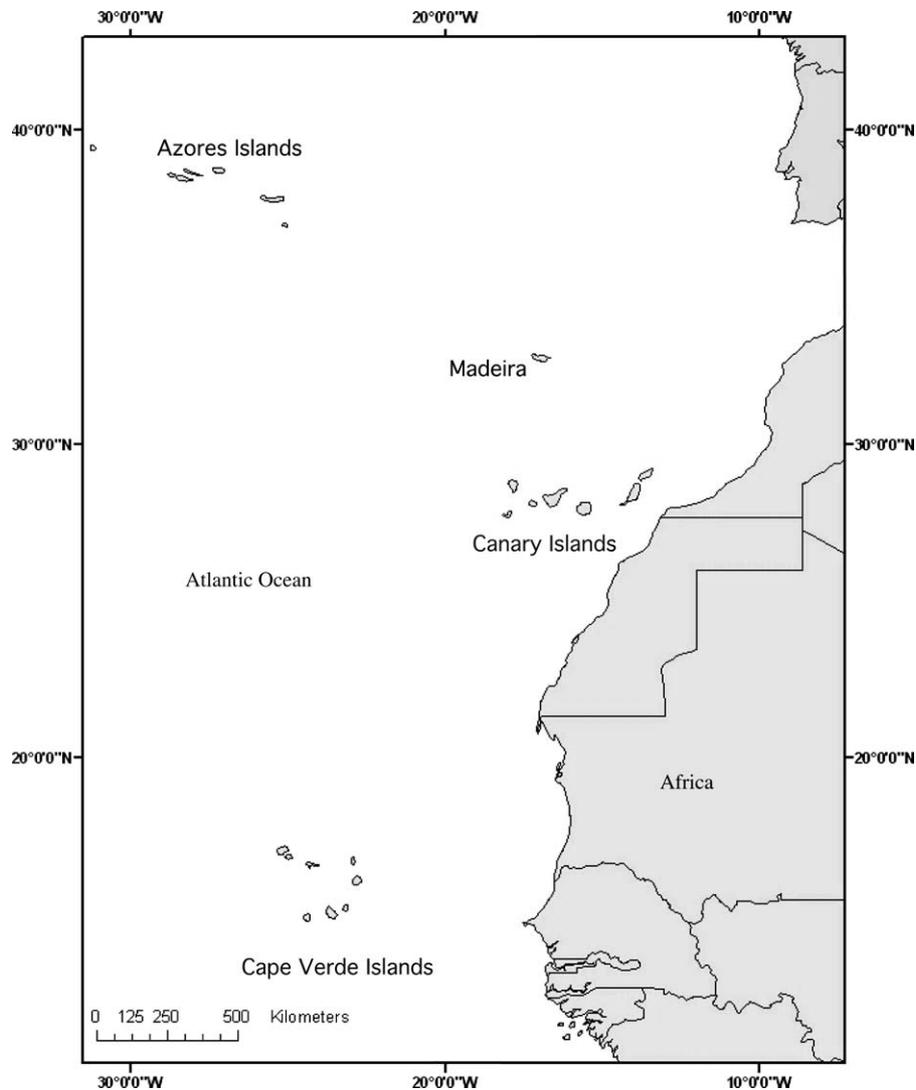


Fig. 1. Map of the Macaronesian Islands and their relative proximity to the African continent.

correspond to the two centers of species diversity (Allan and Porter, 2000; Allan et al., 2003). The clade that includes the Mediterranean species of *Lotus* also includes at least one species, *Lotus campylocladus*, which is endemic to the Canary Islands (Allan et al., 2003). The western North American species of *Lotus* form two separate monophyletic groups, neither of which includes any Macaronesian species from the Mediterranean region (Allan et al., 2003).

In addition to the two centers of diversity, species of *Lotus* are distributed on several island archipelagos. These include the California Channel Islands, the Aegean Islands, and the Macaronesian Islands. The greatest number of insular endemic taxa, however, occurs on the Canary Island archipelago, with approximately 16 species distributed on one or more of the islands (Fig. 2).

To investigate the biogeographic history and taxonomy of Canary Island *Lotus* we examine phylogenetic patterns in the context of the following questions: (1) Are Canary Island *Lotus* monophyletic, implying a single colonization event, or were there multiple colonizations from different continental sources? (2) What was the likely source area or areas from which Canary Island *Lotus* were derived? (3) Are there characteristic patterns of colonization and diversification within the Canary Island archipelago? For example (a) do different species restricted to a single island form monophyletic groups, suggesting a single colonization, or are they the result of multiple colonization events; and (b) is species diversification driven by insular radiation within an island, or geographic isolation via interisland colonization? Finally, we ask whether the current taxonomic classification of Macaronesian *Lotus* is consistent with molecular phylogenetic data.

2. Materials and methods

2.1. Taxonomic sampling

DNA sequence data for the ITS1, 5.8S, and ITS2 regions were obtained for 42 ingroup and 10 outgroup taxa (Table 1). Members of the ingroup include 10 traditionally recognized genera of tribe Loteae s. l., (Loteae + Coronilleae as defined by Polhill, 1981 and Sokoloff, 1998), with extensive taxonomic sampling from the genus *Lotus*. Representatives of *Lotus* include the following four subgenera: *Lotus*; *Tetragonolobus*; *Pedrosia*; and *Rhyncholobus*. The latter two subgenera include species which are either restricted to the Canary Islands (subg. *Rhyncholobus*), or have a predominant Canary Island occurrence (subg. *Pedrosia*). Nearly half (19/40) of the species classified within *Lotus* subgenus *Pedrosia* were included. Eight taxa classified within subgenus *Pedrosia* and that have an exclusive Macaronesian distribution were not available for this study. Two of these eight taxa are Canarian endemics, with the remaining six taxa restricted to either Madeira or the Cape Verde Islands. All four species of *Lotus* subgenus *Rhyncholobus* were included. Three Mediterranean species of *Dorycnium* and a taxonomically controversial species, *L. creticus*, were also included in the data set. *Dorycnium* includes both Mediterranean and Macaronesian species, the latter of which were not available for this study. *Lotus creticus* shares key morphological features with both subgenus *Pedrosia* and subgenus *Rhyncholobus*, but is not classified within either of these subgenera (Kramina and Sokoloff, 1999).

Given the close proximity of the Canary Islands to several continental and insular source areas, we included a broad geographic sampling of species of *Lotus* and

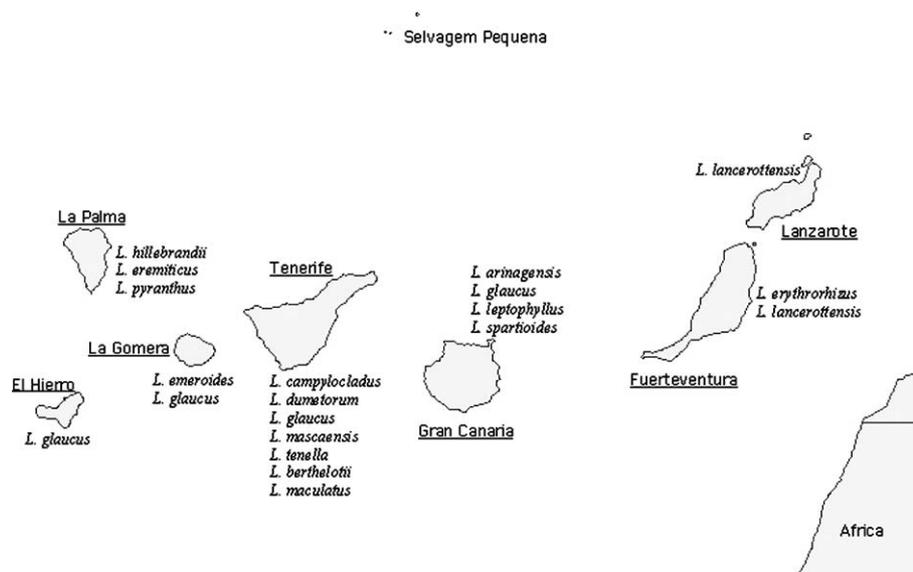


Fig. 2. Map showing the distribution of *Lotus* on the Canary Islands.

Table 1
List of accessions used in phylogenetic analysis of Canary Island *Lotus* and other related Loteae

Taxon	Collection Info.	Geographic distribution	Biogeographic designation/ (Habitat)	GenBank No.
Ingroup genera				
<i>Anthyllis onobrychioides</i> Cav.	A. Ortiz #504; Spain	EUR	EUR	AF450210
<i>Coronilla orientalis</i> Mill. = <i>Securigera orientalis</i> (Miller) Lassen	s.n.; 1996; leg. N. Orlova	EUR	EUR	AF450230
<i>Dorycnium pentaphyllum</i> Scop.	Elias 6472	EUR/MED-AFR	EUR+	AF218501
<i>Dorycnium hirsutum</i> (L.) Ser.	A. Santos	EUR/MED-AFR	EUR+	AY294292
<i>Hammatolobium kremerianum</i> (Coss.) Muell.	D. Podlech 51378	MED-AFR	AFR	AF450233
<i>Hippocrepis emerus</i> (L.) Lassen	Raven 6805, USA: California	EUR/MED-AFR	EUR+	AF218531
<i>Hymenocarpus circinnatus</i> (L.) Savi	Sofajy, s.n., Eurasia	EUR	EUR	AF218504
<i>Kebirita roudairei</i> (Bonnet) Kramina & D. D. Sokoloff	J. Ewald/F. Schuhwerk90/639	SAH-AFR	AFR	AF450200
<i>Lotus</i> subg. <i>Lotus</i>				
<i>L. angustissimus</i> L.	cult. #PI368894	EUR/MED-AFR/ MAC	EUR+	AF450185
<i>L. arabicus</i> L.	cult. #PI214109	EUR/SAH-AFR/ TROP-AFR	AFR	AF450176
<i>L. conimbricensis</i> Brot.	cult. #PI308033	EUR/MED-AFR/ MAC	EUR+	AF450186
<i>L. creticus</i> L.	cult. #PI287858-64i	EUR/MED-AFR/ MAC	EUR+	AF450192
<i>L. ornithopodioides</i> L.	cult., Orel Botanic Grdn #74	EUR/MED-AFR/ MAC	EUR+	AF450205
<i>L. parviflorus</i> Desf.	cult. #PI283615	EUR/MED-AFR/ MAC	EUR+	AF450194
<i>L. weilleri</i> Maire	cult. #PI368910	MED-AFR	AFR	AF450180
<i>Lotus</i> subg. <i>Pedrosia</i> (Lowe) Brand				
<i>L. arenarius</i> Brot.	Podlech 46130	EUR/MED-AFR	AFR	AF218528
<i>L. arinagensis</i> Bramw.	A. Santos, Gran Canaria	CIS (C)	CIS/(CLS)	AY294306
<i>L. assakensis</i> Coss. ex Brand	F. Schuhwerk #90/320	MED-AFR	AFR	AF450204
<i>L. azoricus</i> P. W. Ball	JAO 161-00, Azores	MAC (Azores)	Azores	AY294293
<i>L. campylocladus</i> Webb & Berth.	A. Santos, Tenerife	CIS (T)	CIS/(HCM)	AF450196
<i>L. dumetorum</i> Webb ex R. P. Murray	A. Santos, Tenerife	CIS (T)	CIS/(MON)	AY294294
<i>L. emeroides</i> R. P. Murray	A. Santos, Gomera	CIS (G)	CIS/(MON)	AY294295
<i>L. erythrorhizus</i> Bolle	A. Santos, Fuerteventura	CIS (F)	CIS/(CLS)	AY294296
<i>L. glaucus</i> Sol.	A. Santos, Tenerife	CIS (T, H, C, G and Madeira)	CIS/(CLS)	AY294297
<i>L. hillebrandii</i> Christ	A. Santos, La Palma	CIS (P)	CIS/(MON)	AY294298
<i>L. jacobaeus</i> L.	A. Santos, cult, JAO 190-98	TROP-AFR (Cape Verde Islands)	CV	AY294299
<i>L. lanceroensis</i> Webb & Berth	A. Santos, Lanzarote	CIS/ (L, F)	CIS/(CLS)	AY294300
<i>L. leptophyllus</i> (Lowe) K. Larsen	A. Santos, Gran Canaria	CIS (C)	CIS/(CLS)	AY294301
<i>L. maroccanus</i> Ball	GJA, G31279, Morocco, Africa	MED-AFR	AFR	AF450181
<i>L. mascaensis</i> Burchard	A. Santos, Tenerife	CIS (T)	CIS/(CLS)	AY294302
<i>L. purpureus</i> Webb	A. Santos, cult. JAO 130-99	MAC (Cape Verde Islands)	CV	AY294303
<i>L. spartioides</i> Webb & Berth.	A. Santos, Gran Canaria	CIS (C)	CIS/(MON)	AY294304
<i>L. tenella</i> Lowe	A. Santos, Tenerife	CIS (T)	CIS/(CLS)	AY294305
<i>Lotus</i> subg. <i>Rhyncholotus</i> Monod				
<i>L. berthelotii</i> Masf.	A. Santos, cult. JAO 161-00	CIS (T)	CIS/(MON)	AY294306
<i>L. eremiticus</i> A. Santos	A. Santos, cult. JAO 430-95	CIS (P)	CIS/(MON)	AY294307
<i>L. maculatus</i> Breitfeld	A. Santos, cult. JAO 431-95	CIS (T)	CIS/(CLS)	AY294308
<i>L. pyranthus</i> P. Perez	A. Santos, cult. JAO s.n.	CIS (P)	CIS/(MON)	AY294309
<i>Lotus</i> subg. <i>Tetragonolobus</i> (Scop.) Asch. Et Graebn.				
<i>L. maritimus</i> L.	Skvortsov, s.n., Estonia	EUR/MED-AFR	EUR+	AF218505
<i>L. tetragonolobus</i> L. (= <i>Tetragonolobus purpureus</i> Moench)	Vasak, s.n., Greece, Crete	EUR/MED-AFR	EUR+	AF218506
<i>Ornithopus perpusillus</i> L.	P. M. Uribe-Echebarria #270	EUR/MED-AFR/ MAC	EUR+	AF450226

Table 1 (continued)

Taxon	Collection Info.	Geographic distribution	Biogeographic designation/ (Habitat)	GenBank No.
<i>Scorpiurus vermiculatus</i> L.	Galiano 97/70, Spain	EUR/MED-AFR/ MAC	EUR+	AF218536
<i>Vermifruax abyssinica</i> (A. Rich.) Gillett	Thulin 9304; Yemen	EUR/TROP-AFR	EUR+	AF450235
Outgroups				
<i>Hebestigma cubense</i> (Kunth) Urban	Lavin, 5611	NA		AF450157
<i>Hybosema robustum</i> Sousa & Lavin	M. Sousa et. Al. 13212 (MEXU)	NA		AF450156
<i>Lennea melanocarpa</i> (Schldl.) Harms	Lavin 5787a (TEX)	NA		AF450155
<i>Olneya tesota</i> A. Gray	Lavin 4642 (TEX)	NA		AF450158
<i>Robinia pseudoacacia</i> L.	Allan 263, USA: California	NA		AF218538
<i>Sesbania cannabina</i> (Retz.) Pers.	GenBank accession	AFR/Asia/Australia		AF536351
<i>S. emerus</i> (Aublet) Urban	GenBank accession	Caribbean/Americas		AF536352
<i>S. grandiflora</i> (L.) Pers.	GenBank accession	Cosmopolitan		AF536354
<i>S. tomentosa</i> Hook. & Arn.	GenBank accession	Caribbean		AF536355
<i>S. vesicaria</i> (Jacq.) Elliott	Lavin 6194	NA		AF398761

Geographic distributions as given in the International Legume Database and Information Service (ILDIS) are as follows: EUR, Eurasia including Japan and Taiwan; MED-AFR, mediterranean Africa; SAH-AFR, Sahara Africa; TROP-AFR, Tropical Africa; MAC, Macaronesia (excluding the Canary Islands; CV, Cape Verde Islands; and AZ, Azores); CIS, Canary Islands (C, Gran Canaria; T, Tenerife; F, Fuerteventura; P, La Palma; G, La Gomera; H, El Hierro; and L, Lanzarote); NA, North America (including Mexico and the Caribbean). Abbreviations for the habitat of the Canary Island species are as follows: HCM, High Canarian mountain (1800–2200 m); MON, Montane (500–1700 m); CLS, Coastal and lowland scrub (sea level–500 m). Traditional taxonomy of Loteae (e.g., Ball, 1968; Polhill, 1994) is used, with a few nomenclatural corrections.

closely related genera (Table 1). Geographic distributions of representatives from these 10 genera include Europe, the Mediterranean region, Africa, western Asia, and additional Macaronesian islands (Cape Verde and Azores) where *Lotus* occurs. Outgroup sampling included members of the predominantly tropical, New World tribe Robinieae. Representatives of Robinieae were selected for outgroup comparison based on previous molecular phylogenetic analyses, which strongly support an alliance between Robinieae and Loteae (Hu et al., 2000; Wojciechowski et al., 2000). These studies further identified one member of Robinieae, the genus *Sesbania*, as the weakly supported sister group to Loteae. Subsequent molecular phylogenetic evidence from multiple data sets has led to the redefinition of Robineae such that *Sesbania* is excluded from the robinoid legumes (Lavin et al., 2003). We conservatively combine the information from these different studies by including members of both the redefined Robinieae sensu Lavin et al. (2003) and *Sesbania* (see Table 1).

2.2. DNA extraction and sequence data

Total genomic DNA was extracted from herbarium or silica-dried samples. DNA isolation, polymerase chain reaction, cycle sequencing, and automated DNA sequencing were performed as specified in Allan and Porter (2000), or were slightly modified. Modifications included isolation of DNA from the Canary Island samples using Qiagen (Qiagen, Valencia, CA) spin columns. DNA sequence data of these taxa were collected using the ABI Prism Dye Terminator Cycle Sequencing Kit and visualized using an ABI model

Prism-377 DNA automated sequencer at the Smithsonian Institution's Molecular Systematics Laboratory. DNA of all taxa was sequenced in both directions using the primers (ITS1, ITS2 and ITS4, ITS5) of White et al. (1990).

Raw sequence data from each of the four ITS primers was imported into Sequencher 4.1 where they were edited and combined into a consensus sequence. Consensus sequences were imported into Se-Al ver 1.0 (Rambaut, 1996) where they were manually aligned using sequential pairwise comparisons. Alignment was relatively straightforward except for one section of ITS1 consisting of ca. 100 bp that exhibited considerable sequence divergence. This region was excluded in a test analysis with no changes in topology among Macaronesian *Lotus* (only minor changes elsewhere), and was excluded in the final analysis.

Details regarding sequence length and alignment (e.g., indels), number of informative characters, and pairwise divergence values were determined using PAUP* ver. 4.0b10* (Swofford, 2000) and MacClade (Maddison and Maddison, 2000). Summary data for these features are provided in Table 2. DNA sequence data for all taxa have been deposited in GenBank (see Accession Nos., Table 1). The aligned matrix and trees (Figs. 3 and 6) are available from TreeBASE (<http://www.treebase.org/treebase/>).

2.3. Phylogenetic reconstruction

Phylogenetic analyses were performed using maximum parsimony (MP) and for comparison, a Bayesian method of phylogenetic inference (MrBayes, ver. 2.01;

Hulsenbeck and Ronquist, 2001). Parsimony analysis was conducted assuming unordered character states and equal character weighting. Gaps were treated as missing data and these were excluded from all analyses. Parsimony analyses were completed using PAUP* ver. 4.0b10* and employed a heuristic search strategy with 100 random stepwise-addition replicates, TBR branch-swapping, and MULTREES optimization. Consistency index (CI; excluding uninformative characters) and retention index (RI) were also calculated (Farris, 1989; Kluge and Farris, 1969). Branch support was determined with bootstrapping (Felsenstein, 1985) using a simple addition sequence and 100 replicates, with all other parameters equal to those used in the MP analysis.

The Bayesian analysis was conducted using a general time reversible (GTR + I + G) model [nst = 6; GTR + I + G experimentally determined to be the best fit model using Modeltest ver. 3.06 (Posada and Crandall, 1998)] with gamma distributed rate variation across sites and an initial estimate of equal base frequencies (Jukes and Cantor, 1969). The Markov chain Monte Carlo included four incrementally heated chains where $T = 0.2000$. A preliminary run of 25,000 generations was completed to determine at which generation stationarity of log likelihood values was reached (approximately 10,000). This analysis was followed by an exhaustive run involving 4,000,000 generations, with sample frequency (number of trees saved per generation) set at 10,000 and a burn-in value (number of trees ignored) of 80 (20% of total trees saved). The remaining trees from this analysis [$1 + (\# \text{generations} / \text{sample frequency}) = 321$] were saved and imported into PAUP* where a consensus tree with posterior probability values was generated.

Biogeographical patterns were examined by tracing geographic area onto the phylogeny using MacClade version 4.0. Ten geographic areas were used as character states for assessing the continental origin and biogeographic history of Canary Island *Lotus*. Outgroup areas for *Sesbania* included North America, Caribbean, Caribbean + Americas, Australia, Africa and a worldwide designation for one species (*S. grandiflora*). Outgroup area designation for the five robinoid genera sensu Lavin et al., 2003 was entirely North American. Ingroup areas for *Lotus* corresponded to: Eurasia (including the Mediterranean region), Africa, Canary Islands, Cape Verde Islands, and the Azores. Reconstructions were performed on one of the three trees derived from maximum parsimony analyses (see Results section below).

3. Results

3.1. DNA matrix features and sequence divergence

Length variation of the ITS sequences ranged from 210 to 240 bp for ITS1, 191–245 bp for ITS2 and 423–

467 bp for ITS1 and ITS2 combined (Table 2). The length of the 5.8S region varied from 165 to 167 bp. Aligned sequence lengths for these same regions were 298, 256, 542, and 167 bp, respectively. The number of gaps after alignment was 159 for ITS1, 97 for ITS2, 256 for ITS1 and ITS2 combined, and 6 for the 5.8S region. The G + C content ranged from 46.7 to 69.2 in ITS1, 47.8–66.8 in ITS2, 46.7–67.9 in ITS1 and ITS2 combined, and 50.3–54.6 in the 5.8S region. Sequence divergence values among the 52 taxa ranged from 31.8 to 49.6 in ITS1 and 21.6–42.4 in ITS2. Sequence divergence among the Canary Island taxa ranged from 0.0 to 0.05 in ITS1 with no sequence divergence in ITS2 or the 5.8S region. Sequence divergence among ingroup taxa ranged from 0.0–30.1%, 0.0–30.4%, and 0.0–4.2% (ITS1, ITS2, and 5.8S, respectively).

3.2. Phylogenetic results

The number of variable sites (excluding indels) detected in the ITS region was 214 (Table 2). Of these variable sites, 169 were potentially phylogenetically informative, with 51% of these occurring in ITS1 and 49% in ITS2. The number of constant and autapomorphic sites in the ITS region were 88 and 45, respectively. Maximum parsimony analyses identified 3 trees (1329 steps) with uninformative and gapped characters excluded (CI = 0.493; RI = 0.714) and 91 trees (1443 steps) with uninformative and gapped characters included (CI = 0.533; RI = 0.714). The topology of the strict consensus of these individual sets of trees is identical with respect to relationships among Canary Island *Lotus* and its sister group species. Topological relationships among non-Canarian *Lotus* species varied slightly between the two strict consensus trees, but clades containing these alternative relationships also receive low bootstrap values (see below). Both strict consensus trees identify a monophyletic Canary Island group, which includes the Canary Island species plus one species each from Africa (*Lotus assakensis*) and the Azores (*Lotus azoricus*). *Lotus* subgenus *Pedrosia*, as currently defined, is paraphyletic: subgenus *Pedrosia* plus subgenus *Rhyncholotus* form a strongly supported (bootstrap = 100%) clade (Fig. 3).

The Canary Island *Lotus* group (including *L. assakensis* and *L. azoricus*) is supported by a bootstrap value of 52%. Two nested clades (shaded, A and B) within this group receive bootstrap values of 78% (clade A) and 61% (clade B), respectively. Clade A consists of Canary Island, *L. campylocladus*, which is sister to an unresolved group of Canary Island and African (*L. assakensis*) *Lotus*. Clade B includes Canary Island *Lotus dumetorum* and *L. azoricus* (Azores), which are sister to an unresolved group of Canary Island species, including all four members of *L.* subg. *Rhyncholotus* (Fig. 3). The sister group to Canary Island *Lotus* is weakly supported

(bootstrap = 52%) and incompletely resolved, but comprises a clade containing both African and Macaronesian *Lotus*. The Mediterranean species of *Dorycnium* and the taxonomically controversial species *L. creticus* are more closely related to Eurasian *Lotus* than to Macaronesian *Lotus* (Fig. 3).

Bayesian analysis of the ITS sequences yielded 401 (user-defined) trees based on 4,000,000 generations. Preliminary runs using 25,000 generations resulted in stable likelihood values (value = -6792.75) at approximately generation 10,000. After discarding the first 80 of 401 trees saved from 4,000,000 generations a consensus

tree with posterior probabilities was generated. This Bayesian consensus tree is highly concordant with the consensus tree based on parsimony (Fig. 4). Support values for clades in the Bayesian tree, however, are generally higher than those based on parsimony. This appears to be especially true for deeper divergences in the Bayesian tree. Like the parsimony phylogeny, the Bayesian phylogeny also identifies a monophyletic group (pos. prob. = 87) that includes Canary Island *Lotus*, African *L. assakensis* and *L. azoricus*. The sister groups to this clade are also identical, but with better support and resolution for both African *L. arenarius*

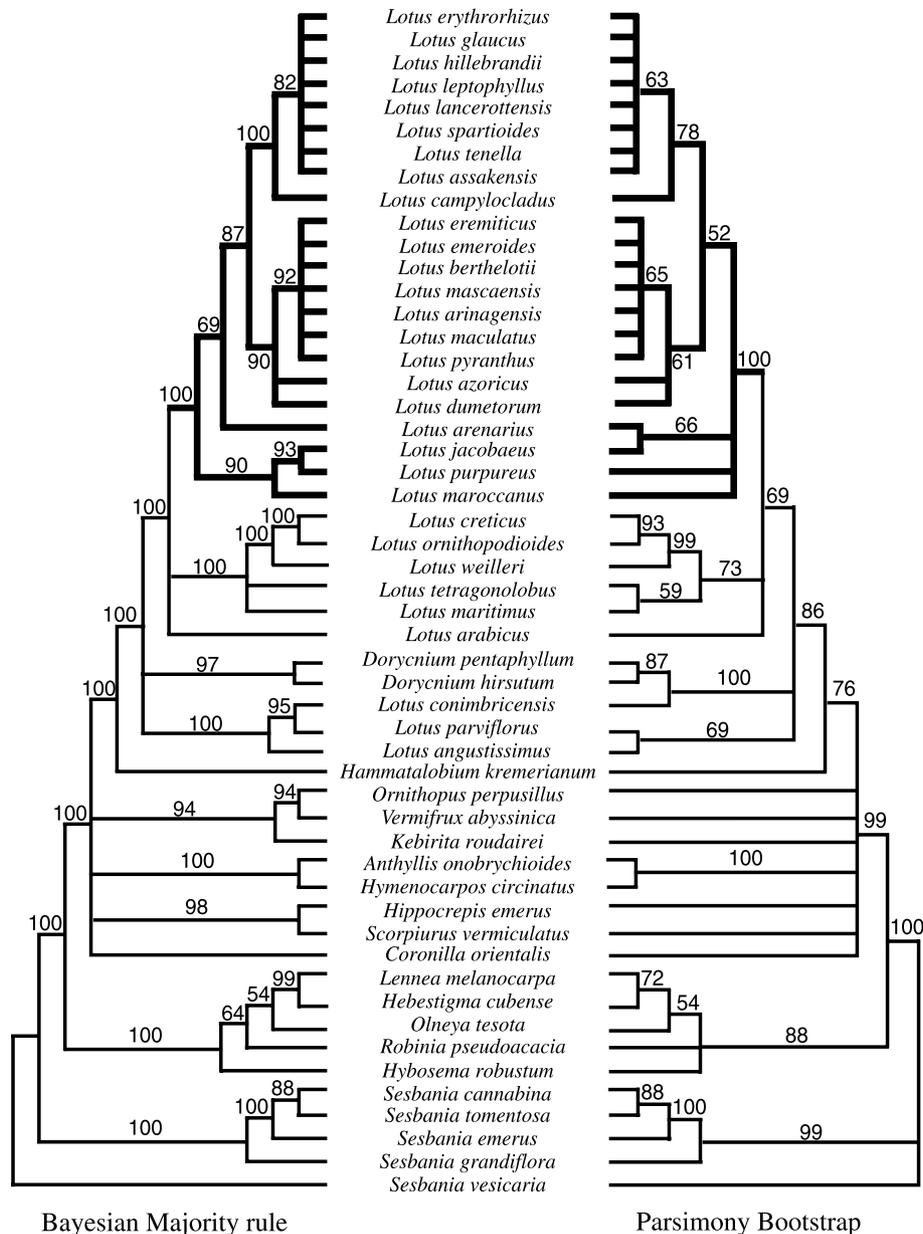


Fig. 4. Comparison of parsimony and Bayesian phylogenetic trees. The maximum parsimony tree is a strict consensus of the three most parsimonious trees (length = 1329, CI = 0.49). Branches in bold indicate *Lotus* subg. *Pedrosia* + *L.* subg. *Rhyncholotus*. Numbers above branches in the Parsimony and Bayesian trees are bootstrap values and Bayesian posterior probabilities, respectively. Note that the Bayesian and parsimony topologies are highly congruent.

and the African/Macaronesian clade identified by parsimony analysis. Relationships among the remaining members of the ingroup are essentially identical to those based on parsimony analyses.

The biogeography of Canary Island *Lotus* was examined using one of three most parsimonious trees (MPTs). All three MPTs had identical topologies for the clade containing species of *Lotus* subg. *Pedrosia* + subg. *Rhyncholotus* (i.e., *Pedrosia* s.l. clade). Given the lack of resolution among the Canary Island species (Fig. 3), we chose a few key taxa from clades A and B to represent

these clades and reconstruct biogeography on a fully resolved tree. Tracing geographic area onto the phylogeny resulted in 786 most parsimonious reconstructions. All reconstructions (using either acc-tran or deltran optimization) unequivocally identify Africa as the source area for Canary Island *Lotus* (Fig. 5, tree length = 15 steps; CI = 0.60; RI = 0.63). This reconstruction also implies one back-dispersal to Africa (*L. assakensis*) and single dispersal events to the Azores (*L. azoricus*) and Cape Verde Islands (*L. purpureus* + *L. jacobaeus*).

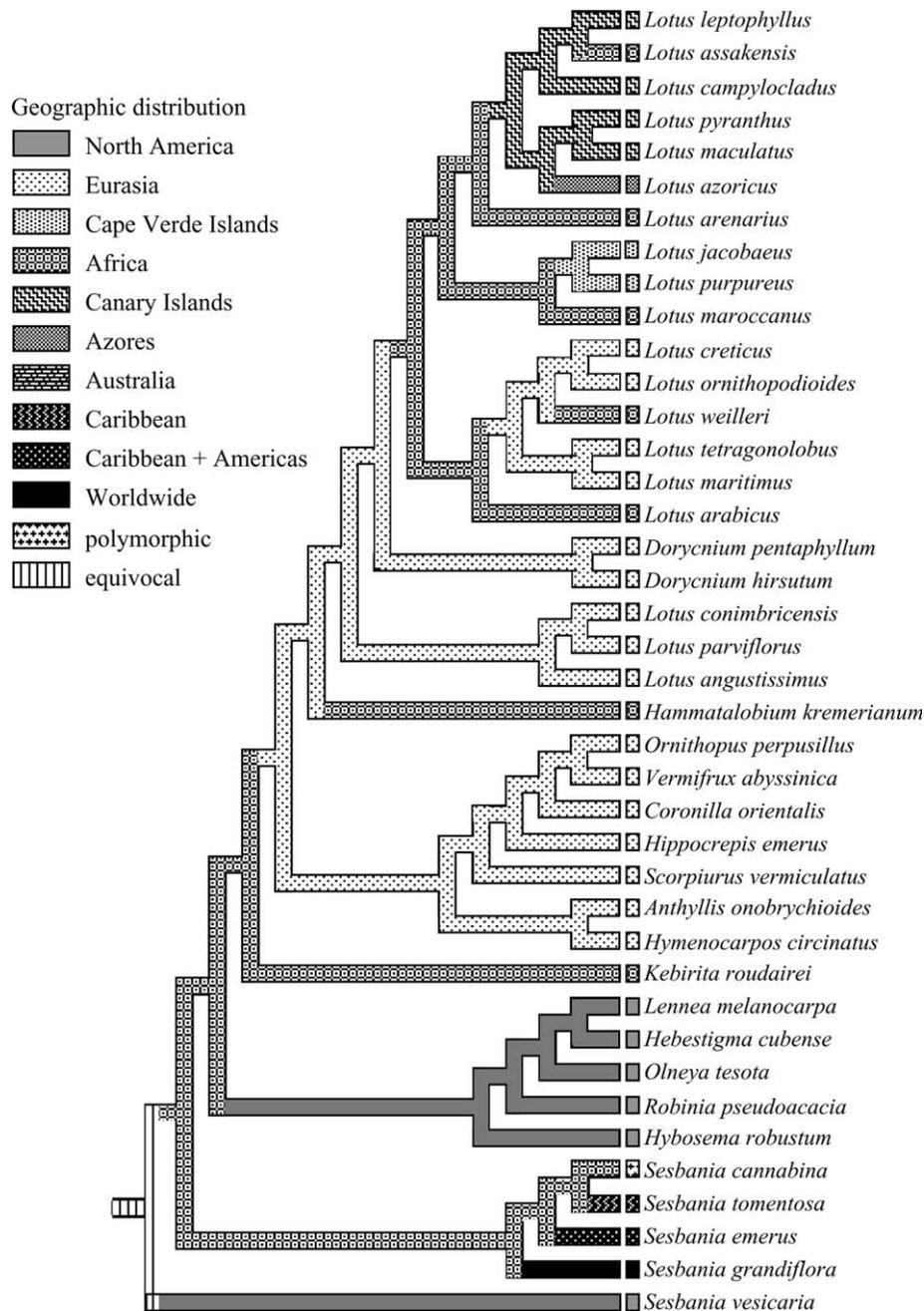


Fig. 5. Most parsimonious acc-tran optimization of the geographic distribution of Canary Island *Lotus* and related genera. Representative taxa from clades A and B (Fig. 3) have been chosen to represent the Canary Island group.

4. Discussion

4.1. Geographic origin of *Canary Island Lotus*

The origins of the Canary Island flora have been investigated using both traditional and molecular-based methods. Bramwell (1972a, 1976) examined patterns of geographic distribution, endemism, chromosome data and the prevalence of woody-life forms, and arrived at the conclusion that most of the insular flora is relictual in origin. Both earlier (Engler, 1879; Meusel, 1965; Takhtajan, 1969) and later authors (Cronk, 1992) have also supported this hypothesis. Molecular based studies, on the other hand, suggest that some elements of the Macaronesian flora are derived from disparate source areas including North America (Panero et al., 1999), Eurosiberia (Clement et al., 1997; Vargas et al., 1999) and southern Africa (Mort et al., 2001). Other molecular-based studies show that a majority of insular plant groups are Mediterranean in origin (Barber et al., 2002; Böhle et al., 1996; Francisco-Ortega et al., 1997a, 1999; Helfgott et al., 2000; Mes et al., 1996; Thiv et al., 1999).

Our most parsimonious reconstruction of geographic area unequivocally identifies (northwestern) Africa as the likely landmass of origin for Canary Island *Lotus* (Fig. 5). This biogeographic reconstruction also identifies a paraphyletic Canary Island assemblage implying a single colonization event to the Canary Islands, followed by back dispersal to Africa, and a single dispersal to the Azores. This expanded Canary Island/African/Azores group is moderately supported by parsimony bootstrap analysis (52%) and well supported in the Bayesian majority rule tree (pos. prob. = 87). Both analyses show good support for the placement of African *L. assakensis* and the Azores endemic, *L. azoricus*, within each of their respective Canary Island clade (clades A and B, Figs. 3 and 4).

Paraphyly of a Canary Island assemblage is somewhat atypical relative to other molecular phylogenetic studies of Macaronesian plants. A majority of studies have identified monophyletic groups of Macaronesian taxa, suggesting a single origin followed by diversification within the insular environment (e.g., *Sideritis*, Barber et al., 2000, 2002; *Bencomia*, Helfgott et al., 2000; *Sonchus*, Kim et al., 1996; *Gonospermum*, Francisco-Ortega et al., 2001; *Pericallis*, Panero et al., 1999; *Crambe*, Francisco-Ortega et al., 1999; and *Argyranthemum*, Francisco-Ortega et al., 1997b). This is not the first time, however, that dispersal from a Macaronesian archipelago to the African continent has been suggested. For example, Mes et al. (1996) found that Macaronesian species of the genus *Aeonium* occupied a basal position relative to other African species, suggesting that dispersal from the islands to the continent is possible. Mort et al. (2001) also found that members of *Aeonium* were paraphyletic, with African *Aeonium* species nested within a Macaronesian clade. Our results contribute to a

growing body of evidence, which suggests that short-distance back dispersal to the African continent is both possible and likely. Finally, our phylogenetic analyses suggest that *L. azoricus* was derived from the same common ancestor that gave rise to the Canary Island assemblage. Our biogeographic reconstruction (Fig. 5) implies long distance dispersal from the Canary Islands to the Azores to account for this taxon, however, parsimony and Bayesian analyses are not sufficiently resolved to rule out the possibility of near contemporaneous colonization of the two archipelagos from Africa. Nevertheless, the idea of the Canary Island archipelago as a stepping-stone for subsequent colonization of more remote Macaronesian islands is a logical scenario.

4.2. Island colonization and diversification

Molecular phylogenetic data have provided considerable insight into understanding patterns of colonization of Macaronesian plants. These studies suggest a mosaic pattern that include both multiple (Cuénod et al., 2000; Hess et al., 2000; Park et al., 2001; Ray, 1995; Vargas et al., 1999) and single colonization events (Böhle et al., 1996; Kim et al., 1996; Francisco-Ortega et al., 1997a; Panero et al., 1999; Barber et al., 2000, 2002). A cladogram of one of our three MPTs suggests a complex pattern of colonization and diversification of *Lotus* on the Canary Islands (Fig. 3). These data suggest that *Lotus* colonized the Islands via a single common ancestor, which gave rise to two separate and relatively recent radiations (clades A and B). One of these radiations (clade A) involved the initial colonization of Tenerife by *L. campylocladus*, followed by colonization and rapid morphological diversification of eight other taxa, seven of which colonized four different islands, and one that was recolonized to Africa (*L. assakensis*). The second event (clade B) may have involved simultaneous colonization of Tenerife (*L. dumetorum*) and the Azores (*L. azoricus*), with subsequent colonization of three other islands (LaPalma, LaGomera, and Gran Canaria). Both of these colonization events involved diversification of taxa in different island environments (two in clade A and three in clade B). The fact that Tenerife appears to be the initial island of colonization in both events suggests that it may have served as a center for dispersal for Canary Island *Lotus*. The idea that Tenerife may have played an important historical role, as a center of dispersal in the Canary Islands, was first suggested by Francisco-Ortega et al. (2002) in their molecular phylogenetic analysis of *Crambe* section *Dendrocrambe* (Brassicaceae). Although our results do not directly test this idea (e.g., through ancestral area analysis), we find the pattern compelling and one that may be resolved with further investigation.

Our assertion of a relatively recent colonization of the islands is supported both by sequence divergence and a

phylogram showing branch length data (Fig. 6). For example, sequence divergence among the island species ranges from 0.0 to 0.05%, and for all species except two (*L. spartioides* = 1 change; *L. campylocladus* = 3 changes) branch lengths are zero (Fig. 6). This scenario of recent colonization was apparently accompanied by rapid morphological diversification as demonstrated by the historical and current acceptance of two distinct subgenera (*L. subg. Pedrosia* and *L. subg. Rhyncholotus*). Our observation of low sequence divergence, coupled with rapid morphological differentiation is consistent with other molecular phylogenetic studies of Macaronesian genera including: *Androcymbium* (Colchicaceae; Caujapé-Castells et al., 1999), *Sideritis* (Barber et al., 2002), the *Astericus* alliance (Francisco-Ortega et al., 1999), and members of *Teline* (Percy and Cronk, 2002).

A primary question for studies of island biogeography focuses on how species diversify within an insular environment. In studies of Macaronesian plants only a handful have identified monophyletic groups of species restricted to a single island (Francisco-Ortega et al., 1996a,b; Kim et al., 1996; Panero et al., 1999; Mort

et al., 2001) and of these, only one plant group, *Tanacetum* (Gonosperminae), has been shown to be the product of an insular radiation (Francisco-Ortega et al., 2001). In most cases, species diversification has been attributed to interisland colonization across similar ecological zones (Francisco-Ortega et al., 2001). One notable exception to this general pattern is found in the island brooms (*Teline*, *Adenocarpus*, and *Genista*; Genisteae: Fabaceae), which show a range of diversification patterns including a possible recent radiation on Tenerife (*Teline canariensis* group), allopatric speciation via interisland colonization (*Teline linifolia* group and *Adenocarpus*), and no diversification for one group (*Genista*), despite being distributed on different Macaronesian archipelagos (Percy and Cronk, 2002).

In the case of Canary Island *Lotus* different species occupying the same island occur in different clades (A and B) and thus do not form monophyletic groups (Fig. 3). For example, six different species occur on Tenerife, but are placed in different clades (three in A and three in B) in the ITS phylogeny. The lack of resolution *within* clades A and B, however, precludes determining whether these species do or do not constitute

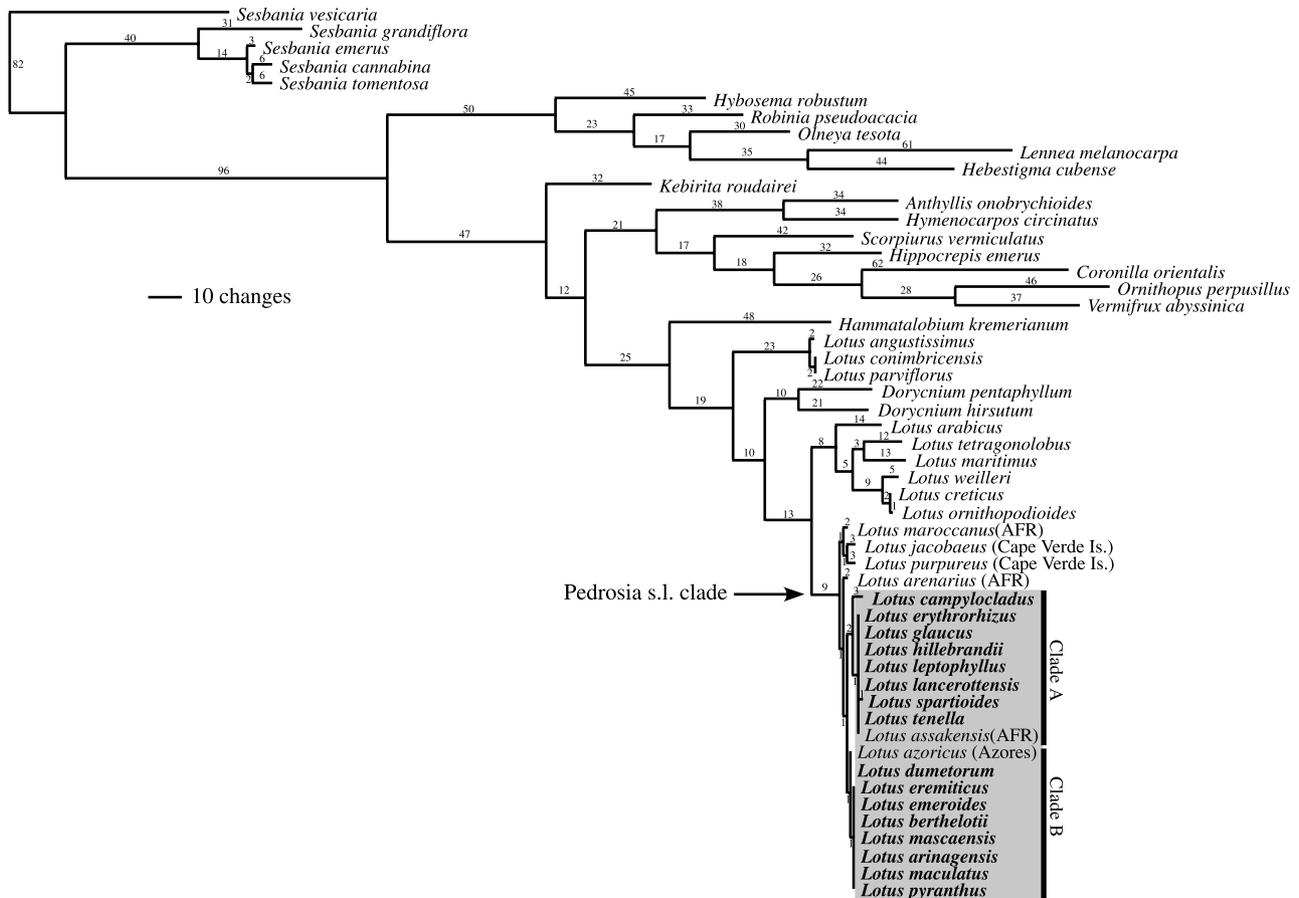


Fig. 6. Phylogram of one of three most parsimonious trees with number of substitutions given above branches. The Canary Island group is highlighted in gray and species endemic to the Canary Islands are in bold type. Other geographic designations are as in Fig. 3.

Table 3
Distribution and habitat of *Lotus* in the Canary Island archipelago

Taxon	La Palma	La Gomera	El Hierro	Tenerife	Gran Canaria	Fuerteventura	Lanzarote
<i>L. arinagensis</i> Bramw.					CLS		
<i>L. campylocladus</i> Webb & Berth.				HCM			
<i>L. dumetorum</i> Webb ex R. P. Murray				MON			
<i>L. emeroideis</i> R. P. Murray		MON					
<i>L. erythrorhizus</i> Bolle						CLS	
<i>L. glaucus</i> Sol.		CLS	CLS	CLS	CLS		
<i>L. hillebrandii</i> Christ	MON						
<i>L. lancerottensis</i> Webb & Berth.						CLS	CLS
<i>L. leptophyllus</i> (Lowe) K. Larsen					CLS		
<i>L. mascaensis</i> Burchard				CLS			
<i>L. spartioides</i> Webb & Berth.					MON		
<i>L. tenella</i> Lowe				CLS			
<i>L. berthelotii</i> Masf.				MON			
<i>L. eremiticus</i> A. Santos	MON						
<i>L. maculatus</i> Breitfeld				CLS			
<i>L. pyranthus</i> P. Perez	MON						

Habitats are abbreviated as follows: MON, Montane; CLS, Coastal and lowland scrub; and HCM, High Canarian Mountain.

independent monophyletic groups. Nevertheless, the fact that the same islands appear in both clades A and B (e.g., Tenerife, Gran Canara, and La Palma) suggests that allopatric speciation via interisland colonization has played an important role in the diversification of Canary Island *Lotus*. We also note that species occupying multiple islands tend to colonize similar ecological environments. For example, *L. lancerottensis* and *L. glaucus*, on two and four islands, respectively, are restricted to coastal lowland scrub within the Canarian archipelago (Table 3). Species that co-occur on the same island (e.g., *Lotus hillebrandii*, *L. eremiticus* and *L. pyranthus* on La Palma and *L. erythrorhizus* and *L. lancerottensis* on Fuerteventura) also occur in similar ecological zones. On large and more ecologically diverse islands such as Tenerife, however, species occupy different habitats (Table 3). This diversification on Tenerife is suggestive of a possible recent radiation, but the ITS data are insufficiently resolved to strongly support this conclusion.

The apparent lack of single island monophyly and the complex colonization patterns observed here may be attributable to one of more of the following factors: (1) the inability of ITS to resolve relationships among recently evolved insular taxa; (2) the close proximity of the islands to one another and to the African continent and; (3) the fact that *Lotus* species are easily and frequently dispersed. The high dispersability of *Lotus* is demonstrated by their extensive cosmopolitan distribution (Isley, 1981) spanning several continents and their frequent occurrence on islands, both near (California Channel Islands; Aegean Islands) and far from continental sources (e.g., widely dispersed in Macaronesia). How *Lotus* achieves such high dispersability, however, is not known. Wind, bird and sea dispersal

may all play a role, but there are no obvious morphological adaptations for dispersal. Other insular legumes such as those of the Genisteae are also widely dispersed throughout Macaronesia (Percy and Cronk, 2002), but like *Lotus*, they exhibit no clear morphological adaptations for dispersal either by air or water. As suggested for members of Genisteae (Percy and Cronk, 2002), the high dispersability of *Lotus* may be accounted for by endozoochorous bird dispersal, but this remains a matter of speculation. Nevertheless, the feasibility with which *Lotus* species colonize islands cannot be disputed, and their ability to exploit a wide range of ecological zones is clearly demonstrated in the Canary Islands.

4.3. Taxonomy

Bentham (1865) was the first to recognize *Lotus* subgenus *Pedrosia*. Using a distinctive feature of the style, Brand (1898) segregated *Lotus* into two groups, those with a forked or toothed style (*Lotus* subgenus *Pedrosia*) and those without (*Lotus* subgenus *Edentolotus* = *Lotus* subgenus *Lotus*). Brand's circumscription included 17 species in two sections, one of which was monotypic. Subsequent authors (Callen, 1959; Gillett, 1959) also recognized *Lotus* subgenus *Pedrosia* as a distinct taxonomic group. Kunkel (1974) included two species (*Lotus berthelotii* and *Lotus maculatus*) under the segregate genus *Heinekenia*. Monod (1980) recognized *Lotus* subgenus *Pedrosia* and *Lotus* subgenus *Pedrosia* section *Heinekenia* (including *L. berthelotii* and *L. maculatus*) using differences in corolla morphology. Numerous cytotoxic studies of Macaronesian *Lotus* have also been conducted (Larsen, 1955, 1958, 1960; Grant, 1965; Bramwell et al., 1971, 1972b; Ortega, 1976,

1979; Gonzales et al., 1993) and chromosome studies in particular have identified two cytological groups: a diploid group ($2n = 14$) and a polyploid group ($2n = 28$); but these do not correspond to the recognition of two distinct subgenera. Studies of cyanogenic glucoside content (Ortega, 1979), however, do support the recognition of *Lotus* subg. *Pedrosia* and *L.* subg. *Rhyncholotus* (= *Lotus* section *Heinekenia* in Ortega, 1979). Current taxonomic classification (International Legume Database Information System at <http://www.ildis.org>) acknowledges *Lotus* subgenus *Pedrosia* and an expanded *Lotus* subgenus *Rhyncholotus*, which, in addition to *L. berthelotii* and *L. maculatus*, includes *L. eremiticus* and *L. pyranthus* (Perez de Paz, 1990).

Data based on the ITS region show that *Lotus* subgenus *Pedrosia* + subgenus *Rhyncholotus* form a strongly supported clade, which is sister to Eurasian species of *Lotus* subg. *Lotus* (Figs. 3 and 4). *Lotus* subgenus *Pedrosia* is therefore paraphyletic in that it includes members of *Lotus* subgenus *Rhyncholotus*. Although it is unclear whether members of *Lotus* subgenus *Rhyncholotus* are themselves monophyletic, their nested position suggests that they could be classified as either *Lotus* subgenus *Pedrosia*, or *Lotus* subgenus *Pedrosia* section *Heinekenia*. This latter classification scheme would be consistent with Monod's (1980) original taxonomic circumscription.

Based on our results, *Lotus* subgenus *Pedrosia* could be considered monophyletic if, and only if it is redefined to include members of *L.* subg. *Rhyncholotus*. Unfortunately, redefining subgenus *Pedrosia* to include subg. *Rhyncholotus* would create a taxonomic group without a clear morphological synapomorphy. This is because one other species, *L. creticus* (subg. *Lotus*) also possesses a toothed style (Kramina and Sokoloff, 1999), but is not included in the clade containing members of *L.* subg. *Pedrosia* + subg. *Rhyncholotus*: *L. creticus* is more closely related to members of *L.* subg. *Lotus* than to members of *Pedrosia* s.l. (Figs. 3 and 4). This result is consistent with an independent origin of the toothed style in at least one other member of *Lotus*. Finally, these data indicate that the Mediterranean species of *Dorycnium* are more closely related to *L.* subg. *Lotus* than to *L.* subg. *Pedrosia*. A complete re-assessment of the taxonomy of *Dorycnium*, however, awaits the inclusion of the Canary Island species (*Dorycnium broussonetii*, *Dorycnium spectabile* and *Dorycnium eriophthalmum*) in subsequent phylogenetic analyses.

5. Utility of ITS and future direction

Molecular phylogenies have and continue to provide new insight into the origin and diversification of the

Macaronesian flora. The utility of the ITS region in particular has been demonstrated in several insular groups and has been instrumental in identifying continental relatives, general patterns of colonization and modes of species diversification (e.g., Barber et al., 2002; Francisco-Ortega et al., 2002; Panero et al., 1999). Our study shows that the ITS region is useful for investigating the geographic origin and taxonomy of Macaronesian *Lotus* (i.e., *Pedrosia* s.l.), but with limited utility for deciphering complex colonization patterns across the Canary Island archipelago. The lack of phylogenetic resolution observed for the insular taxa in this study, however, is mirrored in previous studies (e.g., Barber et al., 2002; Percy and Cronk, 2002) and suggests that the islands were both recently and rapidly colonized without sufficient time for molecular divergence—in spite of considerable morphological diversification in some groups (e.g., *Sideritis*, *Tanacetum*, *Teline*, and *Lotus*). These results suggest that additional molecular markers are needed to more fully resolve the relationships among Macaronesian taxa and to better address instances of putative insular radiation and species diversification. The development of high resolution markers such as the external transcribed spacer (ETS) region of nrDNA (Baldwin and Markos, 1998), which have not been widely employed in Macaronesian studies of plants, but show high utility in studies of other island flora (e.g., the Hawaiian Islands, Nepokroeff et al., 2003; Wright et al., 2001) may assist in generating a more refined picture of the unique evolutionary patterns suggested by ITS phylogenies. Microsatellites, which have been used in studies of the Canary Island fauna (e.g., Richard and Thorpe, 2000), show promise as an independent source of high-resolution markers, although their utility largely depends on having a high rate of mutation relative to migration (Richard and Thorpe, 2001). Nevertheless, a combination of more phylogenetically informative markers such as the ETS region and appropriately evolving microsatellites should prove useful for future studies of the Macaronesian flora.

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