

DIVERSITY AND EVOLUTIONARY HISTORY OF LUPINS – INSIGHTS FROM NEW PHYLOGENIES

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ABSTRACT

Insights into the diversity and evolutionary and biogeographical history of lupins have been limited by confusion about species delimitation, conflicting nomenclature, as well as sparse sampling and lack of resolution in previous phylogenetic studies. Over the last two years we have published a series of more densely sampled (including c. 40% of lupin species), well-resolved, and robustly supported time-calibrated phylogenies, based on DNA sequences from multiple nuclear and plastid genes. In addition, a large number of new chromosome counts have been published for New World species. Here we present an overview of these new phylogenies and the insights they provide into species relationships, the evolution of chromosome number, life history and leaf morphology, in addition to the geographical and temporal patterns of species diversification.

Revised estimates suggest that there are c. 280 species in the genus. While some uncertainty remains as to the precise sister group relationships of the 13 Old World species, current data suggest that the ancestral range for the genus is in the Old World, with at least two independent dispersal events to the New World within the last 5-10 million years. The New World species (excluding the unifoliolate species from Florida) are placed in two robustly supported clades: (i) an eastern, largely lowland $2n = 36$ clade comprising c. 35 species and (ii) a western, largely montane $2n = 48$ clade comprising c. 225 species. These two clades are distributed largely allopatrically with only limited overlap in Texas and the south-central Andes, but both contain ancestral North American and derived South American elements, suggesting at least two independent dispersal events between North America and South America. These new phylogenies also provide clear evidence for two independent evolutionary origins of unifoliolate leaves in Florida and Brazil (congruent with chromosome data), resolving longstanding debate about the relationships of these two groups. Of particular note is the exceptionally high rate of

diversification in the western New World $2n = 48$ clade. In this group a rapid rate of speciation coincides with the transition from annual to perennial life history and colonisation of montane habitats, permitting the exploitation of novel ecological opportunities following recent uplift of the northern Andes. Further work is needed to confirm whether there have been two independent radiations of the western North American perennials and Andean species, or a single larger radiation of the western New World species. Based on these results a number of well-supported clades which are also diagnosable using non-molecular characters, provide the foundation for development of a new infrageneric classification.

KEYWORDS

biogeography, chromosome number, digitate, diversification, *Lupinus*, phylogeny, radiation, unifoliolate

INTRODUCTION

Lupinus comprises c. 280 annual and perennial species. The majority of species occur in the New World, with two main centres of species diversity in western North America (c. 100 species) and the Andes (c. 85 species). In contrast, only 13 species are found in the Old World, predominantly around the Mediterranean. Insights into the diversity and evolutionary and biogeographical history of lupins have been limited by taxonomic confusion about species delimitation, in addition to sparse taxon sampling and reliance on DNA sequence loci lacking sufficient variation resulting in lack of resolution and low levels of clade support in previous phylogenetic studies.

The taxonomy of *Lupinus* has been and still is notoriously problematic. This is due to lack of monographic synthesis and hence consistency of species delimitation across the many regional floristic accounts, and also to the proliferation of names published in the fundamentally flawed Species *Lupinorum* of Smith

(1938-1952). Although progress is being made towards a new monographic taxonomic account, and more sophisticated methods are being brought to bear on species boundary problems for particular clades (e.g., Drummond and Hamilton, 2007), these efforts remain far from complete. This means that species numbers in different groups remain provisional. Even amongst the Old World species which have been thoroughly collected and studied (Gladstones, 1974), new species continue to be discovered (Pascual, 2004). In particular, the taxonomy of the Mexican and Andean species remains in a chaotic state of flux. Intensive field, herbarium and taxonomic research is in progress to delimit the c. 85 Andean species (Hughes and Eastwood, 2006).

Initial phylogenetic studies of *Lupinus* (Käss and Wink, 1997; Ainouche and Bayer, 1999) were hampered by incomplete taxon sampling, especially of South American species, and lack of robustly supported resolution across large portions of the tree. For example, Ainouche and Bayer (1999) included all but one of the 13 Old World species, yet sampled just four of the c. 115 South American species and relied on a single DNA sequence locus, the 5.8S subunit and flanking internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal DNA (nrDNA ITS), which lacks sufficient variation to provide well-supported resolution. Thus, these initial studies have left many questions about the evolutionary history of *Lupinus* unanswered.

Over the last two years a series of more densely sampled (up to c. 40% of *Lupinus*), well-resolved and robustly supported time-calibrated phylogenies, based on DNA sequences from multiple nuclear and plastid genes (Table 1) have been assembled (Hughes and Eastwood, 2006; Drummond, 2008; Eastwood *et al.* in prep.). In addition, a large number of new chromosome counts have been published for New World species (Maciel and Schifino-Wittmann, 2002; Conterato and Schifino-Wittmann, 2006; Camillo *et al.* 2006). Here we present an overview of these new phylogenies and the insights they provide into species relationships, the evolution of chromosome number, life history and leaf morphology, and the geographical and temporal patterns of species diversification. In common with many species-rich plant genera, there are relatively few morphological features for *Lupinus* that can be unambiguously defined and scored as discrete character states. In fact just five characters – life history, leaf morphology, cotyledon form, seed coat surface pattern and chromosome number – have been the focus of detailed comparative morphological studies across the genus. A clear understanding of the evolution of these characters is critical to provide the basis for an infrageneric classification (Eastwood *et al.* in prep.) and to test hypotheses about species diversification processes (Drummond, 2008).

Table 1. Summary of DNA sequence data for *Lupinus*. Nuclear DNA and chloroplast DNA sequence data for *Lupinus*. DNA sequences from GenBank (Käss and Wink, 1997; Ainouche and Bayer, 1999; Ainouche *et al.* 2004; Ree *et al.* 2004; Hughes and Eastwood, 2006; Drummond, 2008) and unpublished data. The phylogenetic analysis of combined data in Fig. 3 was based on a concatenated matrix of all these sequences plus outgroup taxa, excluding specimens for which only ITS was available. The number of included, variable, and parsimony informative sites were calculated for each genomic region after removing outgroup taxa and ambiguously aligned positions.

	<i>nucDNA</i>				<i>cpDNA</i>			
	ITS ¹	LEGCYC1A ²	LEGCYC1B ²	GPAT1 ³	trnS-trnG ⁴	trnT-trnL ⁴	trnL intron ⁴	trnL-trnF ⁴
Sequences	223	144	52	110	93	93	112	19
Named species	109	81	33	70	60	60	66	17
Aligned sites	633	1327	1443	589	960	674	507	499
Included sites	633	1159	1281	534	765	619	492	379
Variable sites	169	430	280	231	119	110	61	27
Parsimony informative sites	131	266	116	114	73	62	44	18

¹The 5.8S subunit and flanking internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal DNA.

²Two copies of *CYCLOIDEA*-like *LEGCYC1* regulatory genes have been characterised in Genistoid legumes (Hughes and Eastwood, 2006).

³Two copies of GPAT (Glycerol-3-phosphate acyltransferase) have been isolated and sequenced from *Lupinus* (Eastwood *et al.* unpubl. data)

⁴Four non-coding plastid intron and intergenic spacers sequenced by Drummond (2008), with additional sequences from Ainouche *et al.* (2004) and Ainouche and Misset (unpubl. data).

METHODS

The volume of DNA sequence data for *Lupinus* has grown dramatically in recent years (Table 1). Different loci have been sequenced for different but overlapping sets of species, reflecting the objectives of the independent studies that generated these data. For example, plastid sequencing has focused on North American taxa (Drummond, 2008) and nuclear loci on South American taxa (Hughes and Eastwood, 2006; Eastwood *et al.* unpubl. data). The ITS region of nrDNA has been sequenced for more taxa than any other (Table 1), but lacks sufficient sequence divergence to build a well-resolved tree (Käss and Wink, 1997; Ainouche and Bayer, 1999; Ainouche *et al.* 2004) and occurs in large tandemly repeated arrays which can cause paralogy problems in phylogenetic analyses (Bailey *et al.* 2003). In order to provide an effective and succinct overview of these new phylogenetic datasets, we have selected three phylogenetic trees (Figs 1-3) based on different combinations of DNA sequence loci and produced using different analytical methods to describe what is currently known about the evolutionary history of lupins: (i) parsimony analysis of a combined data set comprising nrDNA ITS, nuclear *CYCLOIDEA*-like regulatory, *LEGCYCIA*, and nuclear Glycerol-3-phosphate acyltransferase, GPAT1, gene sequences for 84 *Lupinus* species (Table 1) to investigate the evolution of leaves, chromosomes and life history (Fig. 1); (ii) a time-calibrated phylogeny based on a combined nrDNA ITS and *LEGCYCIA* data set including 98 species to look at geographic and temporal patterns of species diversification (Fig. 2), and (iii) analysis of a data set comprising all the available plastid and nuclear data listed in Table 1 using a super-matrix approach, to assemble the most densely sampled phylogeny for the genus so far, to be used as the basis for a new infrageneric classification of the genus (Fig. 3). The aim in each case has been to maximise taxon sampling for the different analyses, some of which are more susceptible to missing data than others. Details about specific analyses are provided in the figure captions. Full information about taxon sampling, collection vouchers, laboratory and analysis methods are provided in Hughes and Eastwood (2006), Drummond (2008) and Eastwood *et al.* (in prep.).

RESULTS AND DISCUSSION

NEW PHYLOGENIES

The new phylogenetic trees for *Lupinus* (Hughes and Eastwood, 2006; Drummond, 2008) and the three summary trees presented here (Figs 1-3) are all broadly congruent. The different analyses identify the same set of robustly supported groups, giving confidence to these as the basis for a new infrageneric classification (Fig. 3). However, some conflict is apparent amongst the differing hypotheses of sister group relationships from different data sets, especially at the base of the tree (Figs 1-3), leaving some uncertainty about the precise

relationships of the Old World species and a need for caution in interpreting the history of dispersal (see below). Furthermore, the relationships among the numerous Andean species remain largely unresolved. The existence of an eastern New World clade has been postulated by previous authors (Dunn, 1984; Planchuelo, 1984) and hinted at in several older phylogenies (Käss and Wink, 1997; Ainouche and Bayer, 1999), but the full extent of this clade spanning both North and South American elements and including the South American unifoliolate species (see below) is only now apparent (Fig. 2). Furthermore, the affinities of the North American unifoliolate species to Old World groups rather than the South American unifoliolate species are revealed for the first time (Figs 1-3).

LEAVES

One of the most prominent morphological features in *Lupinus* is the occurrence of both digitate and unifoliolate leaves. While the majority of *Lupinus* species have digitate leaves, there are c. 19 unifoliolate species. These occur in two disjunct geographic areas in the south-east U.S.A. (3- 4 species) (Dunn, 1971) and eastern South America (15 species) (Planchuelo and Dunn, 1984; Monteiro and Gibbs, 1986). Ever since Agardh (1835) and Bentham (1859) established their informal groups *Foliiis integris* and *Simplicifoliae* respectively, the unifoliolate species have been universally considered to comprise a natural group of closely related species (Agardh, 1835; Bentham, 1859; Dunn, 1971; Monteiro and Gibbs, 1986). Dunn (1971) went further and proposed that the North American unifoliolate species were derived from the South American unifoliolate species via long distance dispersal and subsequent diversification. However, the only evidence to support this idea has been the striking morphological similarity in leaves and stipules (e.g. between *L. sellowianus* from southern Brazil and *L. villosus* from Florida), and the relationships of these species have never previously been explicitly tested via phylogenetic analysis. This apparently straightforward categorisation into digitate and unifoliolate masks two important complexities. First, a subset of species with digitate adult leaves have unifoliolate juvenile leaves (Planchuelo and Dunn, 1984). Second, one South American species, *L. paraguariensis* has both digitate and unifoliolate adult leaves on the same shoot, representing a superficially 'intermediate' transition state (Planchuelo and Dunn, 1984).

Recent phylogenetic analyses (Hughes and Eastwood, 2006; Eastwood *et al.* in prep.) included a substantial sample of unifoliolate species from both South America and Florida. These new phylogenies show unambiguously that digitate leaves are the ancestral condition and that unifoliolate leaves evolved twice independently within the genus (Fig. 1, nodes A and B), contrary to all previous ideas about leaf evolution (Dunn, 1971, 1984; Planchuelo and Dunn, 1984; Gross, 1986). Under this scenario there is no need

to invoke a long distance dispersal event between eastern South America and Florida as proposed by Dunn (1971). It is also notable that the South American unifoliolate species are nested within a wider group of species with unifoliolate juvenile leaves and that *L. paraguariensis*, the 'intermediate' species with both

digitate and unifoliolate adult leaves, is sister to the unifoliolate group (Fig. 1). This suggests a set of multiple evolutionary transitions towards greater reduction, or progenesis, of leaf parts, culminating in the very unusual reduced sessile unifoliolate leaves of species like *L. parvifolius*.

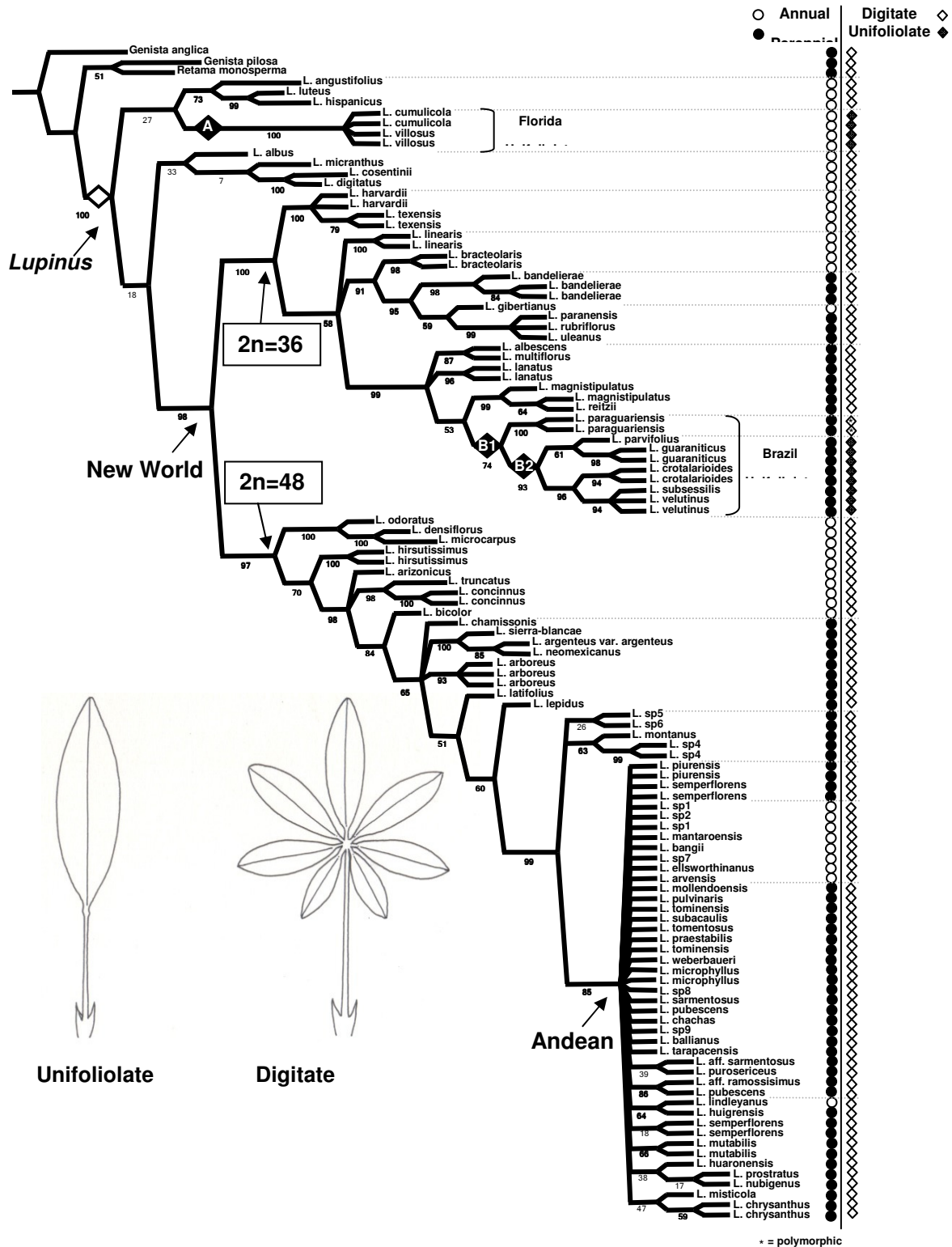


Fig. 1. Evolution of leaves and life history in *Lupinus*. Strict consensus of 82,909 equally parsimonious trees from combined analysis of nDNA ITS, *GPAT1* and *LEGCYCIA*. Bootstrap values are shown beneath branches. Nodes A and B represent independent transitions from digitate to unifoliolate leaves in North and South America respectively.

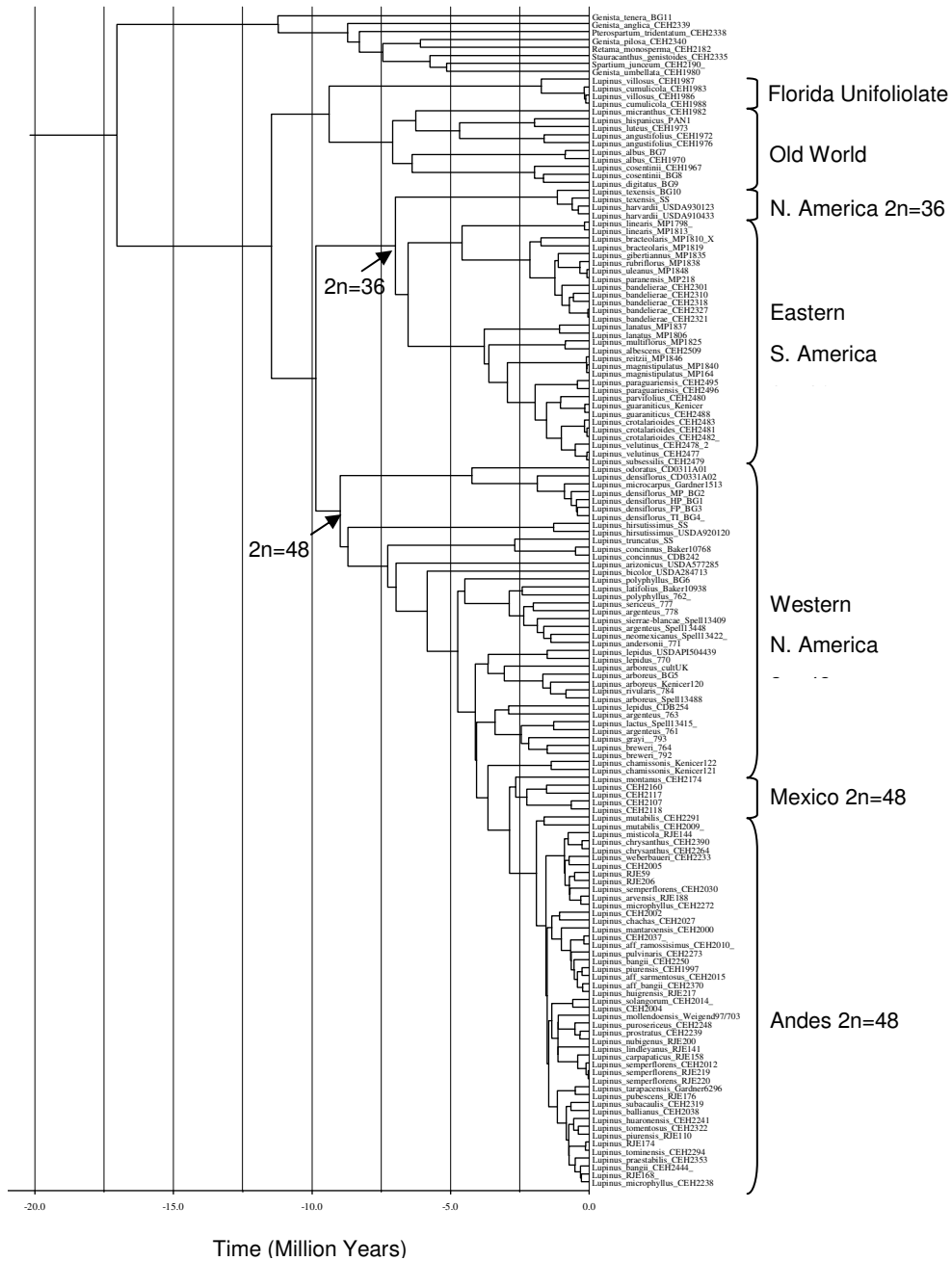


Fig. 2. Time calibrated phylogeny of *Lupinus*. This is based on a relaxed-clock Bayesian analysis of the combined ITS and *LEGYC1A* data sets. The tree was calibrated based on the estimated age of the *Lupinus* stem node from the legume wide analysis of matK sequences using 11 fossil calibrations (Lavin *et al.* 2005).

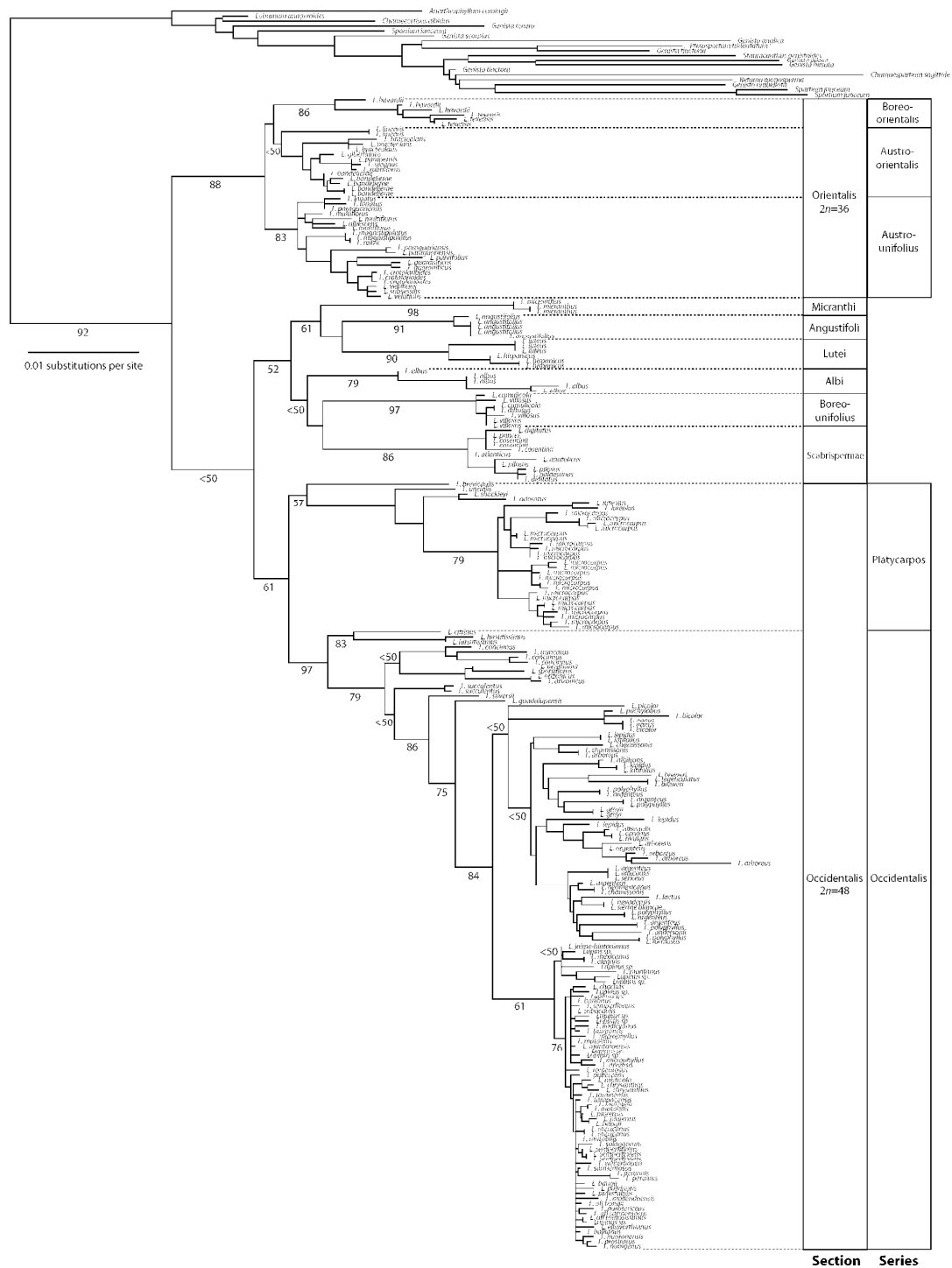


Fig. 3. Infrageneric classification of *Lupinus* (formally presented in Eastwood *et al.* in prep.) based on maximum likelihood analysis of combined sequence data from nuclear DNA (ITS1 + 2, *LEGCYCA1*, *LEGCYCIB*, *GPAT1*) and chloroplast DNA (*trnS-trnG*, *trnT-trnL*, *trnL intron*, *trnL-trnF*). The topology shown here represents the single tree with the greatest log-likelihood (ln L = -28276.7832) from multiple independent searches of a concatenated data matrix (*Lupinus* = 105 spp., 234 specimens; outgroup = 15 spp., 18 specimens) under the GTR+G model in GARLI0.96b8. Support for clades (100 non-parametric bootstrap replicates) is indicated next to branches leading to key nodes.

The habitats where the North and South American unifoliolate species grow show striking ecological similarities. Both groups of unifoliolate species grow in lowland, subtropical, fire-prone grassland/Cerrado or fire-prone pine barren habitats and comprise populations of sparsely scattered individuals quite different from the generally gregarious populations of other lupins. These similarities suggest common adaptive significance of the unifoliolate condition, possibly to promote rapid growth in fire-prone habitats.

CHROMOSOMES

Diverse chromosome numbers have long been documented for the Old World species ($2n = 32-52$), but until recently cytogenetic data on New World lupins, and especially South American species, were limited to just a few species. Furthermore, data on chromosome numbers have not been assessed in a phylogenetic context. A series of recent cytogenetic studies were the first to provide chromosome counts for a substantial set of South American species (Maciel and Schifino-Wittmann, 2002; Conterato and Schifino-Wittmann, 2006; Camillo *et al.* 2006). In contrast to the great variability in chromosome numbers amongst the 13 Old World species, the accumulated data on chromosome numbers for 88 New World species show that these are much more uniform in terms of chromosome number with $2n = 36$ for nearly all the eastern lowland South American and Texan species (the exceptions being *L. bracteolaris* and *L. linearis* with $2n = 32, 34$) and $2n = 48$ (with occasional $2n = 96$ polyploids) for the very large cohort of western New World species (Conterato and Schifino-Wittmann, 2006).

The most striking finding from these recent studies is the congruence between chromosome number and the two large and robustly supported New World lineages – the eastern lowland New World group is consistently $2n = 36$ (with only minor exceptions – see above), while the large group of western, mainly montane New World species is uniformly $2n = 48$ (Fig. 1). The existence of these two separate eastern and western New World lineages has long been suspected (Dunn, 1984; Planchuelo, 1984), but is now confirmed and shown to be congruent with chromosome number (Fig. 1). The congruence between chromosome number and leaf morphology is also striking. All the South American unifoliolate species have $2n = 36$ chromosomes, while the North American unifoliolate species *L. cumulicola* and *L. villosus* have $2n = 52$ in line with their placement as sister to the Old World species (Fig. 1).

LIFE HISTORY

Diverse life history strategies are apparent amongst lupins. Both annuals and perennials are common, with the perennial species encompassing both herbaceous and woody species, including prostrate dwarf mat-forming alpine plants, clump-forming rosettes lacking shoots, unusual stem rosettes reminiscent of giant tropicalpine Senecios or Lobelias, woody shrubs and even small treelets (Drummond, 2008; Hughes and

Eastwood, 2006). The evolution of life history in terms of annual and perennial habit was investigated in detail by Drummond (2008) who showed that perennials are derived from annuals multiple times within the genus and that this shift is correlated with montane environments, most notably within the western New World group. Thus annual species tend to occur in more arid and strongly seasonal lowland habitats, while perennials predominate in more mesic, upland environments. Wider taxon sampling confirms that perennial life history has evolved from annual habit independently multiple times within the genus, and that reversals from perennial to annual life history have also occurred (Fig. 1). It is notable that these reversals also appear to be correlated with ecology. For example, although the Andean clade is predominantly composed of perennial species, the handful of Andean species that show annual life history, such as *L. mollendoensis* and *L. lindleyanus*, grow at low to mid elevations on the drier Pacific slopes of the Andes.

GEOGRAPHIC AND TEMPORAL PATTERNS OF SPECIES DIVERSIFICATION

There has also been much speculation about the geographical and temporal patterns of species diversification within the genus. In fact, hypotheses about the ancestral area for the genus have encompassed all the main areas where extant species occur today – the Old World Mediterranean region (Cristofolini and Chiapella, 1984; Käss and Wink, 1997), North America (Plitmann, 1981) and South America (Dunn, 1984; Gross, 1986; Gladstones, 1998). The new phylogenies show evidence of strong geographical structure with individual clades largely restricted to single geographical areas (Fig. 2). However, even with the availability of significant new DNA sequence data sets, the sister group relationships along the backbone of the tree remain weakly supported, at times conflicting amongst different analyses (Figs 1-3). Despite these uncertainties, the available evidence suggests that the ancestral area for the genus lies in the Old World and that estimated divergence times for New World/Old World nodes are less than 10 million years (Fig. 2) (Hughes and Eastwood, 2006; Drummond, 2008). This scenario indicates at least two long distance dispersals from the Old World to the New World (Fig. 2), as previously proposed by Käss and Wink (1997) and Gladstones (1998). The New World species (excluding the unifoliolate species from Florida) are placed in two robustly supported large clades: (i) an eastern, largely lowland $2n = 36$ clade comprising c. 35 species and (ii) a western, largely montane $2n = 48$ clade comprising c. 225 species (Figs 1 and 2). These two clades are distributed largely allopatrically with only limited overlap in Texas and the south-central Andes but both contain ancestral North American and derived South American elements, suggesting at least two independent North to South America dispersals ca. 7 and < 2 million years ago (Fig. 2). Of particular note, the recent and exceptionally high rate of species diversification in the western New World $2n = 48$ clade is as rapid as for any

land plant group documented to date (Hughes and Eastwood, 2006; Drummond, 2008). This shift to faster rates of diversification coincides with the transition from annual to perennial life history and colonisation of cooler wetter montane habitats (Drummond, 2008), as well as the creation of novel ecological opportunities following recent uplift of the northern Andes (Hughes and Eastwood, 2006). Further work is needed to confirm whether there have been two independent western New World radiations (Drummond, 2008) or a single larger radiation (Hughes and Eastwood, 2006), but the western New World lupins are without doubt remarkable for their very recent and extremely rapid diversification, which has occurred on an intercontinental scale that spans Alaska to the south-central Andes.

INFRAGENERIC CLASSIFICATION

Alongside chaotic taxonomy, another disconcerting feature of *Lupinus* is that there is currently no complete or acceptable infrageneric classification. Furthermore, none of the many, often contradictory and partial attempts to provide a classification have been based on a well sampled phylogeny. The new 'total evidence' phylogeny based on combined analysis of all available DNA sequence data (Fig. 3) alongside data about the evolution of major morphological characters provide the basis for a new infrageneric classification of the genus (Eastwood *et al.* in prep.), that combines natural morphological groups with diagnosability of Sections and Series within the genus. This classification (Fig. 3) will be formally presented by Eastwood *et al.* (in prep.).

CONCLUSIONS

This summary paper outlines the current state of *Lupinus* phylogenetics, which has been transformed using new DNA sequence data in terms of taxon sampling, resolution and clade support over the last five years. There is now good scope to generate a 'complete' *Lupinus* phylogeny by adding more species to the existing DNA sequence data sets, filling in the missing data gaps caused by differing samples for various genomic regions, and generating new DNA sequences that are variable enough to resolve relationships amongst the recently evolved western New World species.

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