Towards the 21st Century

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MOLECULAR PHYLOGENY AND PHYLOGEOGRAPHY OF
THE GENUS Lupinus

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ABSTRACT

The phylogeny and phylgeography of lupins were reconstructed in relation
to the Genistae and other Papilionoideae using nucleotide sequences of the
chloroplast gene rbcL and nuclear ITS 1+2 DNA. Lupinus forms a monophyletic
clade that shares common ancestry with other Genistae. Both groups represent the
most advanced taxa of the Papilionoideae. Within the genus Lupinus, the rough-
seeded lupins of the Old World represent a monophyletic clade that is well separated
from smooth seeded lupins. The phylogenetic trees imply that lupins evolved in the
Old World and that the New World was colonised in several waves. One line leads
to the South American species of Atlantic origin, other lines to the North American
species and South American species of Andean distribution. Since evolution of
lupins apparently took place within the last 10 million years, colonisation of the
New World can not be explained by plate tectonics. Therefore, it must have
occurred via migration across the Bering Strait or via long distance dispersal.

INTRODUCTION

The genus Lupinus L. is a rather large genus within the Leguminosae. The
size of the genus and the wide geographical distribution of its taxa, and relatively
uniform morphological features have always complicated a thorough taxonomic
analysis. Furthermore, an integration of the genus Lupinus in the tribe Genistae
remained ambiguous. This has led to the exclusion at least from part of the
Genistae (Rothmaler, 1944), the constitution of a tribe together with Argyrolobium
(Hutchinson, 1964) or the separation as a monogeneric subtribe (Bisby, 1981). A
number of hypotheses have been put forward for the phylogography of the genus
Lupinus. Lupinus should have evolved directly from Sophoroid ancestors in South
America (Dunn, 1971), or out of Crotalarieae with South America as a centre of
origin (Gross, 1984; Dunn, 1984). Alternatively, Lupinus should have derived from
Thermopsisideae (Thermopsis) with a North American origin. In this case the rough
and smooth seeded groups could represent single or separate evolutionary lines
(Plitmann, 1981). Also an evolution out of primitive Genistae in the
Mediterranean region has been considered (Cristofolini & Chiapella, 1984), or
lupins, together with the rest of the Genistae, should have derived from
Thermopsisideae (Turner, 1981). It has also been speculated that after an early

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evolution of the genus a further separation of species was caused by plate tectonics (Nowacki & Jaworski, 1978).

In this paper, we have reconstructed the phylogeny and phylogeography of lupins from nucleotide sequences of the chloroplast gene rbcL and nuclear ITS1 +2 DNA. These are useful molecular markers to analyse the evolutionary past of plants (Soltis et al., 1992; Chase et al., 1993; Clegg, 1993; Olmstead & Palmer, 1994).

METHODS


RESULTS AND DISCUSSION

The evolution of the Papilionoideae from Sophora to Lupinus is shown in Figure 1 based on nucleotide sequences of the rbcL gene that is a useful marker to reconstruct relationships between genera and families (Chase et al., 1993; Doyle, 1994). Part of the Sophoreae built the base of the Papilionoideae. Molecular data imply that the Sophoreae are an artificial conglomerate of polyphyletic origin, but none could possibly be a direct ancestor to Lupinus. The next cluster branching off the evolutionary line leading to the lupins contains members of Sophoreae, Podalyrieae and Thermopsidae. Genetic data do not confirm that Thermopsis or other "woody temperate Thermopsidae" were direct ancestors of the Genistaeae. Genistaeae and Crotalarieae represent the most advanced groups in all reconstructions (Kass and Wink, 1995, 1996, 1997a,b). The number of nucleotide substitutions implies that neither group can be ancestor of the other. Lupinus and the rest of the Genistaeae are a sister taxon to the Crotalarieae and form a monophyletic clade. The taxonomic rank of Lupinus and the Genistaeae seems to be best described by Bisby (1981), who assigned subtribus rank to Lupinus and grouped the rest of the Genistaeae without assigning a taxonomic rank.

Evolution within the Lupinus complex is shown in Figure 2 based on nucleotide sequences of nuclear ITS 1+2 DNA that shows more variation than the rbcL gene. Considering their position in the phylogenetic tree, we suggest that the Old World lupins are the central group, from which the South American lupins of eastern distribution may have branched off early.

Molecular data clearly shows that the rough-seeded lupins are a closely related monophyletic group. Plitmann (1981) marked that the differences between the rough seeded species are mainly quantitative. Surveys based on alkaloid (Carstairs et al., 1992, Wink et al., 1995), flavonoid (Williams et al., 1983) and protein data (Salamanowicz & Przybylska, 1994) also supported the uniformness of this group. The rough-seeded species were thought to be ancestral to North American species when flavonoid patterns were evaluated (Williams et al., 1983, Plitmann & Heyn 1984). Molecular data indicate that they were separated before the evolution of the North American species, and that smooth seeded species stand
closer to the North American taxa, as suggested from seed characters and alkaloid data (Plitmann, 1981; Plitmann & Pazy, 1984).

Figure 1. Phylogenetic tree of members of the Papilionoideae based on rbcL sequences using Cercis siliquastrum as an outgroup. Analysis by Maximum Parsimony; Phylogram of the single most parsimonious tree of a Brânc & Bound search (Length 251 steps, min/max length 184-546 steps; CI 0.733, HI 0.267, RI 0.815, RC 0.597). Branch lengths are proportional to the number of inferred changes between taxa (indicated above each branch).

The marked differences of rough and smooth seeded lupins, which are evident from molecular data, can also be seen in serological (Cristofolini, 1989), protein (Salamanowicz and Przybylska 1994) and flavonoid data (Williams et al.,
1983). However, the occurrence of lupinine or multiflorine and derivatives cut through these groups (Wink et al., 1995).

![Phylogenetic tree of the genus Lupinus](image)

**Figure 2.** Phylogenetic tree of members of the genus *Lupinus* based on ITS sequences using *Erinacea anthyllis* as an outgroup. Analysis by Maximum Parsimony; Phylogram of one of six most parsimonious trees of a Branch & Bound search (Length 124 steps, min/max length 104-259 steps; CI 0.839, HI 0.161, RI 0.871, RC 0.30). Branch lengths are proportional to the number of inferred changes (which correspond to the number of nucleotide substitutions) between taxa (indicated above each branch).

The smooth-seeded species are far more heterogeneous than the rough-seeded ones. The close relation of *L. luteus* and *L. hispanicus* was never in
question, but *L. angustifolius* has to be counted to this group according to molecular data (and flavonoid pattern; Williams et al., 1983). *L. albus* and *L. micranthus* seem to be (not very closely) genetically related. Their alkaloid pattern also shows similarities (Wink et al., 1995), but their flavonoid pattern differs (Williams et al., 1983). Genetically, *L. micranthus* differs most from all other Old World lupins, confirming serological data (Cristofolini, 1989).

Only part of the New world lupins has been covered in our present study, but at least three lines are apparent.

1) The South American species of eastern origin.

2) The North American species and South American species of western distribution and

3) At least the "microcarpi" (Smith. 1944) ("platycarpos"; Watson, 1873) group with *L. microcarpus* and *L. densiflorus*.

While Watson did not further subdivide the North American species, some groups of Smith are confirmed by the molecular data (like the "micranth"-group; *L. nanus, L. polycarpus*). Others (like the "arborei"-group of shrubby taxa) are not well supported. Flavonoid data (Nicholls & Bohm, 1982) and alkaloid data do not give a clear pattern either. The incongruence of alkaloid and flavonoid patterns with the molecular data can be explained by the fact that secondary metabolites constitute adaptive traits since they function as defence or signal compounds (Wink, 1988, 1992, 1993). Convergence is common for adaptive traits (but not for molecular data) which obscures a phylogenetic analysis (Wink et al., 1993).

**Phylogeography of *Lupinus***

The cluster standing basal to the *Genisteae* and *Crotalariaeae* comprises taxa of mainly Old World distribution (except part of *Thermopsis* and *Baptisia*). This fact and the derived position of the North American lupins does not favour a New World origin of *Lupinus* although species numbers are much higher in the New than in the Old World. An Old World origin of *Lupinus* together with the *Genisteae* seems much more likely. The position of *Podalyrieae* and *Crotalarieae*, which have their radiation centres in Africa, would suggest that the origin might not have been Sino-Himalayan, but rather African.

From evolutionary lines (Fig. 1, 2) and divergence times (Table 1) it could be concluded that lupins reached the American continent several times from their Old World origin. Migration via the Bering Strait could have been one way for the European species to reach North America until recent times (correlating well with a divergence time of about 3-4 mio year: Table 1). However, it is not certain how the South American species with eastern distribution ("Atlantic species") reached the New World. A long range dispersal (for example by strong hurricanes), as Dunn (1971) suggested for the simple leaved lupins of North America, could be a plausible but speculative possibility. It seems certain however, that the present distribution cannot be explained by continental drift because lupins are much too young; i.e. continents were already well separated when lupins evolved (see times in Table 1). For a more detailed discussion see Käss and Wink (1997a).
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Table 1. Divergence times of different groups of the Papilionoideae (Mio years) based on ITS1+2 sequences.

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<tr>
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<th>ITS1</th>
<th>ITS2</th>
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<tr>
<td>Lupinus Old World/</td>
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<td>North American species</td>
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<td>Thermopsisae/Sophoreae</td>
<td>~33</td>
<td>~34</td>
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