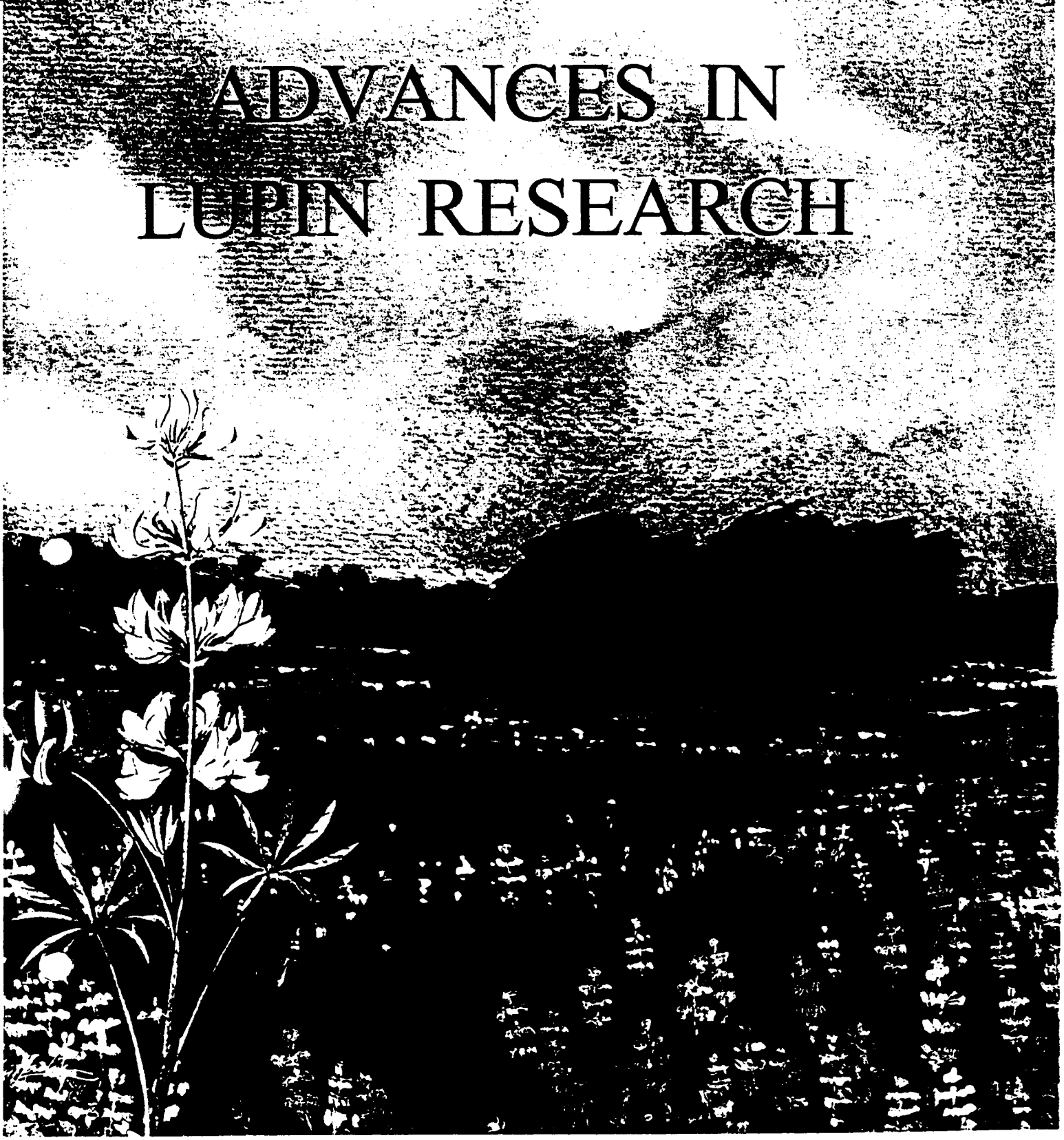
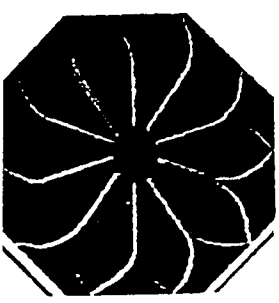
ADVANCES IN LUPIN RESEARCH





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MOLECULAR PHYLOGENY OF LUPINS

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ABSTRACT

It has been assumed that lupins originate from South America and that they have conquered North America and the Old Would. In order to elucidate the phylogenetic origin of lupin and their migrations we have started to employ methods of molecular biology, such us Polymerase Chain Reaction (PCR) and DNA sequencing. Using these methods we have amplified and sequenced a chloroplast gene, rbcL and a nuclear gene, pPLZ2 from more than a dozen lupin taxa. Comparing the DNA sequences obtained, a preliminary phylogenetic tree will be presented. The alkaloid profiles of these lupin will be discussed for comparison.

INTRODUCTION

As a consequence of the rapid development of methods for the analysis of DNA the use of molecular data in studies on plant systematics has become more and more important. From the point of molecular evolution the chloroplast genome is of special interest. It is generally inherited maternally, which avoids the problems of polyploidy. There are no multigene families and normally it does not contain insertions or deletions. One of the genes of major interest located on the cloroplast genome is the rbcL gene which codes for the large subunit of the ribulose-1,5-biphosphate carboxylase, a key enzyme in photosynthesis. Until now about 600 rbcL sequences have been determined which allow a first reconstruction of plant evolution (Chase et al. 1993).

MATERIAL AND METHODS

Extraction of plant DNA

The plant material is homogenized using mortar and pestle, then incubated in CTAB (Cetyltrimethylammonium bromide) buffer that breaks cell walls and releases cellular contents. After incubation the DNA is precipitated with ethanol and purified.

Polymerase Chain Reaction (PCR)

Normally a genome contains a small number of copies of a gene which makes it impossible to determine the nucleotide sequence directly. For the amplification of DNA PCR has become a revolutionary method. Single stranded oligonucleotides (primers) that hybridize with the beginning and the end of the gene are synthesized. A thermostable polymerase then completes the strands starting from the primer (Mullis *et al.*, 1986). This reaction runs automatically in a thermocycler and the number of DNA copies is doubled at each step. After amplification the DNA is purified and may either be sequenced directly or cloned for further multiplication.

Cloning into plasmid vectors

Plasmid DNA is cut by Hinc ll, a restriction enzyme that creates blunt ends. Foreign DNA (e.g., the purified PCR product) is ligated to the plasmid and used to transform *E.coli* cells. After E.coli has grown to the desired density the plasmid DNA is harvested by lysis of the cells, precipitation and purification.

Sequencing of DNA

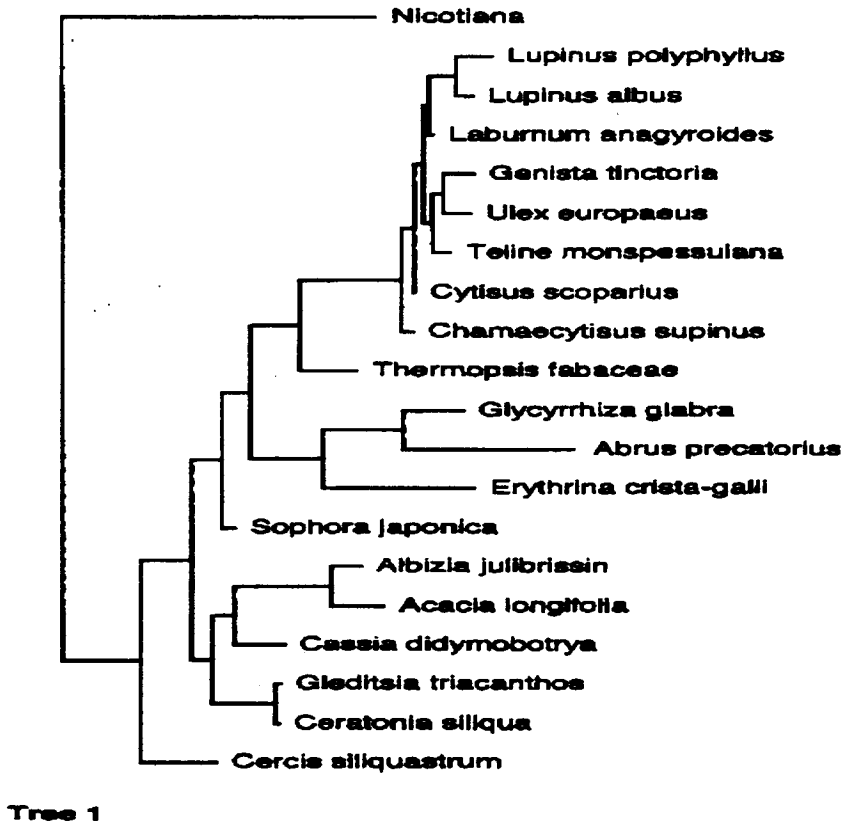
Sequencing is done by the dideoxy sequencing method according to Sanger. Again a DNA-polymerase synthesizes the complementary strand of the sequence starting from a primer. Here we need one reaction for each nucleotide and we end up with four fractions of single stranded DNA all starting from the primer but ending at all possible positions of the nucleotide in question. These are separated by electrophoresis on an acrylamide gel and as they are radioactive a typical band pattern appears after exposition on an x-ray film.

Analysis

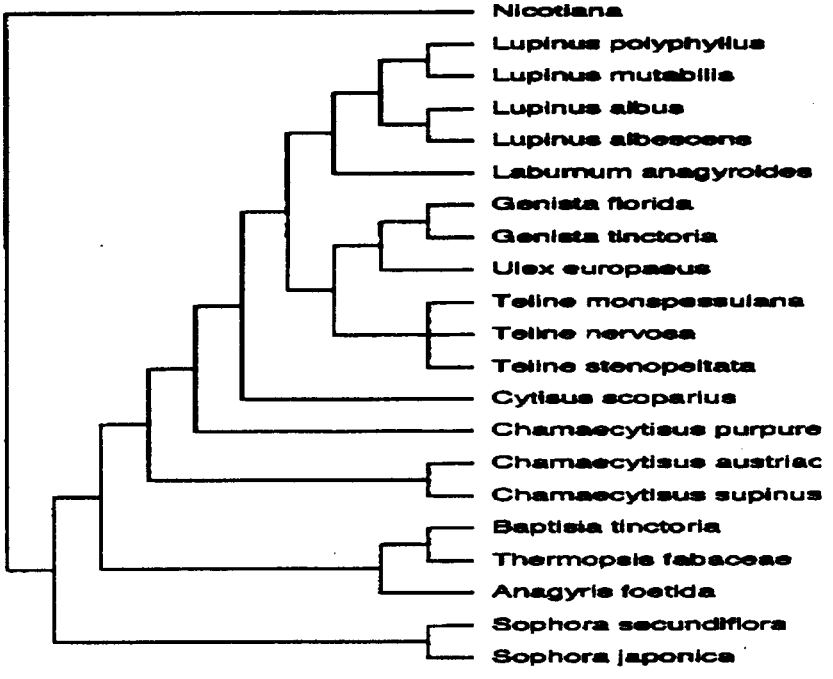
Reading of the films and alignment of the sequences is done manually. Tree construction is done by PAUP 3.1 (Phylogenetic Analysis Using Parsimony)(Swofford 1993), a computer program which employs maximum parsimony methods.

RESULTS AND DISCUSSION

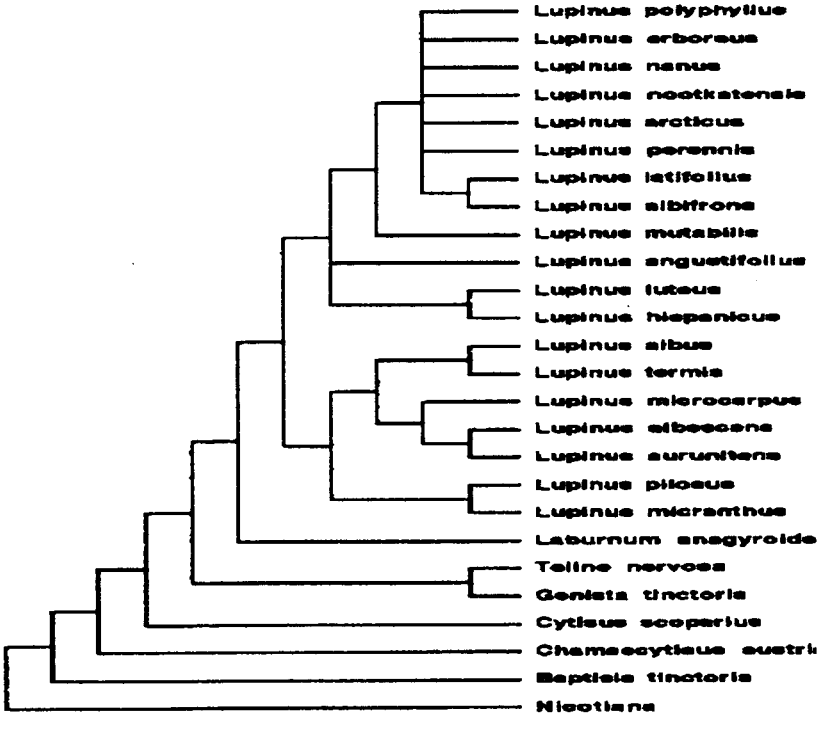
DNA of various taxa of Leguminosae, especially of those of the genus *Lupinus* was amplified by PCR, cloned and sequenced. Sequence data were used to reconstruct phylogenetic trees employing heuristic methods (Swofford 1993).



Tree 1 provides an overview of the overall phylogeny of *Leguminosae* which differentiates the established three subfamilies: *Papilionoideae* (*Lupinus* - *Sophora*), *Mimosoideae* (*Albizia* - *Acacia*) and Caesalpinioideae (*Cassia* - *Cercis*).



Tree 2



Tree 3

Tree 2 includes taxa only (i.e. of the *Papilionoideae*) which contain quinolizidine alkaloids. The taxa are genetically related, though the tribes form clearly distinguished phylogenetic units: - *Genisteae* (*Lupinus* - *Chamaecytisus*), *Thermopsideae* (*Baptisia* - *Anagyris*) and *Sophoreae* (*Sophora*). Within the *Genisteae* the genera *Lupinus, Laburnum,* the *Genista* complex, the *Cytisus/Chamaecytisus* complex are recognized and confirmed by rbcL-phylogeny. *Teline,* which was formerly included in the genus *Genista, is* closely related

to *Genista,* but obviously distinct. The separation of *Cytisus, Chamaecytisus* and *Genista* as "good" genera is supported by our data.

Tree 3 is a preliminary reconstruction of *Lupinus* phylogeny. We can clearly distinguish the lupins from different continents: North America *(L. nanus - L. albifrons),* Europe/Africa *(L. angustifolius - L. albus)* and South America *(L. albescens - L. microcarpus). L. albus is* separated in 2 subspecies: *L. albus graecus* and *L. albus albus.* The latter is often called synonymously *L. termis.* Our data show identical rbcL sequences for both subspecies, indicating that subspecies level is justified. *Lupinus luteus* and *L. hispanicus* are genetically closely related which was also assumed from phytochemical and morphological characters. *L. mutabilis* shows no affinities for the group of South American lupins, but seems to be related to North American taxa. It might be speculated that *L. mutabilis* has been introduced to South America by early *Homo sapiens.*

All lupins produce quinolizidine alkaloids (Wink 1993). Typical alkaloids for *L. albus, L. pilosus, L. micranthus* and the South American species are multiflorine and derived alkaloids (albine, hydroxymultiflorine, etc.). This group of lupins is also recognized as phylogenetically related by our rbcL-analysis (Tree 3). Lupanine-type alkaloids are characteristic for the New World taxa ranging form *L. polyphyllus* to L. *mutabilis.* Interestingly, the European *L. angustifolius* share some of the chemical and genetical characters. As a preliminary conclusions we suggest that phytochemical patterns and rbcL-data seem to be in fair agreement.

The genus *Lupinus* represents a relatively old genus of monophyletic origin. Old World and South American species are closer related to each other than they are to the North American species sugesting a common origin. North American species are closely related and seem to have migrated via Europe or South America to North America at a later stage. We intend to enlarge the spectrum of taxa in the future and to include nuclear genes in our analysis. These data should finally elucidate the evolutionary history and migration patterns of lupins.

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