Chloroplast DNA characters, phylogeny, and classification of Lathyrus (Fabaceae)[1]

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Mapped cpDNA restriction site characters were analyzed cladistically and the resulting phylogenetic hypotheses were used to test monophyly and relationships of the infrageneric classification of *Lathyrus* (Fabaceae) proposed by Kupicha (1983, *Notes from the Royal Botanic Garden Edinburgh* 41: 209–244). The validity of previously proposed classification systems and questions presented by these classification schemes were explored. Two cpDNA regions, *rpo*C (*rpo*C1, its intron, part of *rpo*C2, and their intergenic spacer) and IR⁻ (*psb*A, *trn*H-GUG, part of *ndh*F, and their intergenic spacers), were analyzed for 42 *Lathyrus* and two *Vicia* species. PCR (polymerase chain reaction) amplified *rpo*C and IR⁻ products digested with 31 and 27 restriction endonucleases, respectively, resulted in 109 potentially informative characters. The strict consensus tree suggests that several of Kupicha's sections may be combined in order to constitute clades. The widespread section *Orobus* and the South American section *Notolathyrus* should be combined. Section *Lathyrus,* characterized by a twisted style, should either include sections *Orobon* and *Orobastrum* or be redefined as three sections, one of which is characterized by a 100 base pair deletion in the IR⁻ region. Finally, a weighted parsimony analysis positions sections *Clymenum* (excluding *L. gloeospermus*) and *Nissolia,* both with phyllodic leaves, as sister sections. The affiliation of *Lathyrus gloeospermus* (section *Clymenum*) remains problematic.

Key words: classification; Fabaceae; inverted repeat; *Lathyrus*; mapped restriction site polymorphism; molecular phylogeny; polymerase chain reaction (PCR); *rpo*C.

The genus *Lathyrus* L. (Fabaceae; Vicieae) consists of ~160 species (summarized by Allkin et al., 1986; new species added by Nelson and Nelson, 1983; Broich, 1986, 1987; Yu-Jian and Ren-Xian, 1986; Maxted and Goyder,

1988; Isely, 1992; Plitmann, Gabay, and Cohen, 1995). *Lathyrus* is distributed throughout the temperate regions of the Northern Hemisphere with 52 species in Europe, 30 species in North America, 78 species in Asia, and 24 species extending into tropical East Africa and 24 species into temperate South America (Kupicha, 1983; Allkin et al., 1985; Goyder, 1986). The main center of diversity is the eastern Mediterranean region, with smaller centers in North and South America (Kupicha, 1983; Simola, 1986). *Lathyrus* species occur in a diversity of habitats, including open woods, forest margins, meadows, pastures, fields, slopes, marshes, seashores, sand dunes, and roadsides. Both annual and perennial species of *Lathyrus* occur, many of which have a climbing or sprawling habit using simple or branched tendrils. *Lathyrus* exhibits a typical bee-pollinated papilionoid flower, which may be yellow, orange, red, purple, violet, bluish, or white.

Kupicha's (1983) morphology-based monograph represents the only worldwide treatment of the genus. Kupicha proposed an infrageneric classification with 13 sections (Table 1) and discussed the more important previous infrageneric *Lathyrus* classifications of Godron (1848), Boissier (1872), Bässler (1966), Davis (1970), and Czefranova (1971; Fig. 1). These classifications are based mainly on morphological characters, which are interpreted in a classical taxonomic way without applying explicit phenetic or cladistic methods of analysis. In addition, they only include European and Asian species. Since Kupicha's revision, a phenetic analysis of morphological characters of 54 Turkish *Lathyrus* species has been conducted (Dogan, Kence, and Tigin, 1992). This study supported Kupicha's sections *Orobus, Lathyrostylis,* and *Clymenum* but disagreed on the circumscription of the remaining sections (Fig. 1).

TABLE 1. Kupicha's (1983) 13 *Lathyrus* sections with number of species and approximate distributions.

Section	No. species	Distribution
Aphaca (J.Mill.) Dumort.	2	Europe, north Africa, and southwest and central Asia
Clymenum (J.Mill.) DC. ex Ser.	3	Mediterranean area
Lathyrostylis (Griseb.) Bässler	20	Europe, northwest Africa, southwest Asia, and Russia
Lathyrus	33	Europe, north Africa, southwest Asia, Turkmenistan, Uzbekistan, and Russia
Linearicarpus Kupicha	7	Europe, north and eastern Africa, southwest Asia, and Russia
Neurolobus Bässler	1	Western Crete
Nissolia (J.Mill.) Dumort.	1	Europe, northwest Africa, Crimea, Caucasia, Turkey, and Iraq
Notolathyrus Kupicha	24	South America except *L. pusillus,* which extends into southeast North America
Orobastrum Boiss.	1	Mediterranean region, Crimea, Armenia, Georgia, and Azerbaijan
Orobon Tamamsch.	1	Crimea, Caucasia, eastern and northern Turkey, and northern Iran
Orobus (L.) Godr.	54	Europe, northwest Africa, former Soviet Union, Asia, Japan, and North and Central America
Pratensis Bässler	6	Europe, northwest Africa, west to central Asia, and Himalayas
Viciopsis Kupicha	1	Southern Europe, western Turkey, and northwest Africa

Besides morphological data, other character complexes have been used to study infrageneric structure and evolution of species of the genus *Lathyrus,* but none of them have resulted in overall classification schemes for the genus. Anatomy, cytology, and enzyme electrophoresis have been useful in defining closely related species (Simola, 1968a; Brunsberg, 1977; Hossaert and Valero, 1985; Plitmann, Heyn, and Weinberger, 1986; Roti-Michelozzi and Bevilacqua, 1990; Godt and Hamrick, 1991a, b, 1993; Valero and Hossaert-McKey, 1991). Chemical compounds such as anthocyanins, flavonoids, and non-protein amino acids have been surveyed for potential use in *Lathyrus* systematics, but chemical characters seem to be most useful at the generic level in the Vicieae (Pecket, 1959, 1960; Przybylska and Nowacki, 1961; Bell, 1962, 1964, 1966; Brunsberg, 1965; Przybylska and Rymowicz, 1965; Simola, 1966, 1968b, 1986).

In summary, previous *Lathyrus* classification schemes and the numerous papers on various aspects of *Lathyrus* taxonomy and biology present hypotheses that have yet
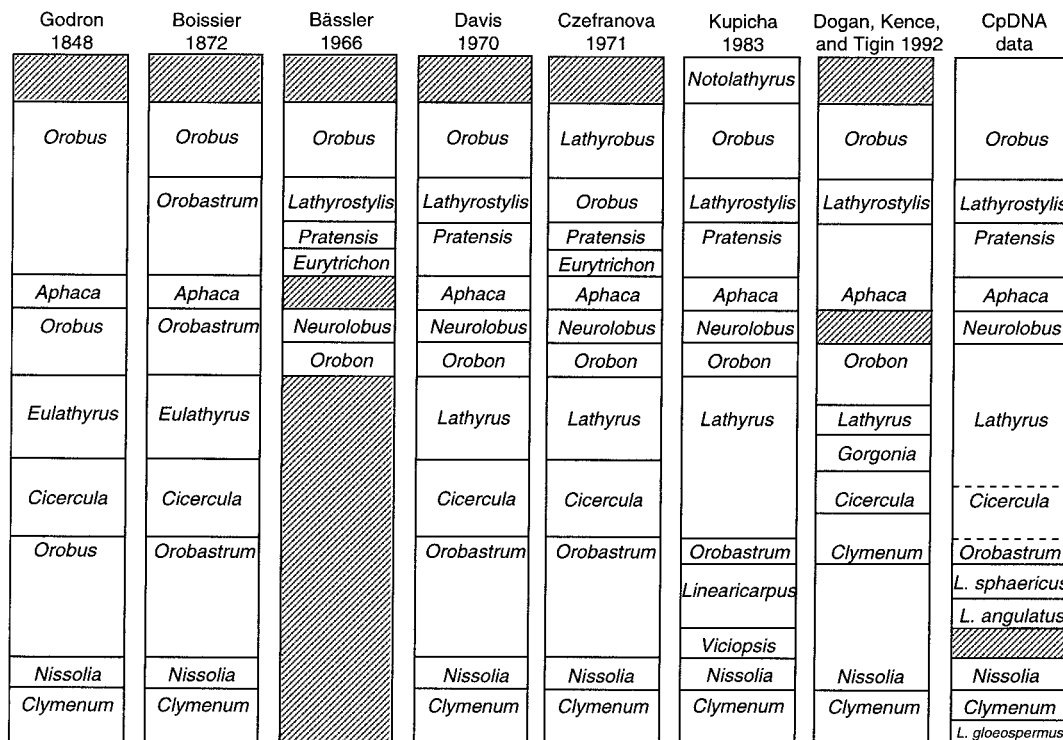
Fig. 1. Comparison of eight *Lathyrus* classification systems, six of which are redrawn from Kupicha (1983, Table 1), one is published since then (Dogan, Kence, and Tigin, 1992), and the last is a preliminary classification based on cpDNA restriction site data from this study. Boissier's (1872) taxon *Orobus* is a genus and his genus *Lathyrus* consists of sections *Orobastrum, Eulathyrus, Cicercula, Aphaca, Nissolia,* and *Clymenum.* In the classification of Bässler (1966) sections *Orobus, Lathyrostylis, Pratensis, Eurytricon, Neurolobus,* and *Orobon* constitute subgenus *Orobus.* Czefranova (1971) ascribes sections *Lathyrobus, Orobus, Pratensis, Eurytrichon,* and *Neurolobus* to subgenus *Orobus,* sections *Orobon, Orobastrum,* and *Lathyrus* to subgenus *Lathyrus,* and *Cicercula, Aphaca, Nissolia,* and *Clymenum* to four monotypic subgenera. Dogan, Kence, and Tigin's (1992) sections *Orobus* and *Lathyrostylis* constitute subgenus *Orobus,* and sections *Aphaca, Nissolia, Orobon, Gorgonia, Clymenum, Cicercula,* and *Lathyrus* are united in subgenus *Lathyrus.* Shaded areas represent groups that were not included.

to be tested and they raise questions about relationships that remain to be answered. Determining whether *Lathyrus* and its sections are monophyletic can direct future monographic work of this economically important genus. Additional unanswered questions include: (1) are the South American *Lathyrus* species part of the holarctic flora element in the Andes and does *Lathyrus* follow a dispersal pattern where South American species are ultimately derived from North American lineages, as predicted from the boreotropics hypothesis (Cleef, 1979; van der Hammen and Cleef, 1986; Simpson and Todzia, 1990; Lavin and Luckow, 1993)? and (2) do the phyllodic leaves found in sections *Clymenum* and *Nissolia,* but which are uncommon in legumes in general, have a single or multiple origins within *Lathyrus*? In order to test the previous classification systems and to answer the questions they raise, new methods of analysis and new data sources can supplement classical analyses of morphological, anatomical, and chemical data.

Molecular data are important sources that have not yet been explored in *Lathyrus* systematics. Restriction site data from the cpDNA locus *rpo*C were phylogenetically informative in *Astragalus* L. (Liston, 1992; Liston and Wheeler, 1994). *Lathyrus* and *Astragalus* both belong to the derived clade of temperate legumes, which share the synapomorphic character state of lacking one copy of the cpDNA inverted repeats (Lavin, Doyle, and Palmer, 1990; Liston, 1995). This suggests that the method useful in *Astragalus* can be expected to solve the same kind of problems within *Lathyrus*. The cpDNA region where one copy of the inverted repeat was lost (Lavin, Doyle, and Palmer, 1990) has been further characterized by Liston (1995) and Ding et al. (1995). This region, termed IR⁻, has not previously been used as a target for restriction enzyme digests and phylogenetic analysis of the resulting restriction site characters. The IR⁻ fragment includes genes of various expected nucleotide substitution rates (Olmstead and Palmer, 1994) in addition to intergenic spacers, which are expected to be more variable than coding regions. Based on previous studies and expected nucleotide substitution rates, molecular data derived from restriction endonuclease digests of PCR amplifications of the two cpDNA fragments, *rpo*C and IR⁻, should be potentially useful in reconstructing phylogenetic relationships of the genus *Lathyrus*. The cpDNA restriction site characters derived from *rpo*C and IR⁻ were analyzed cladistically and the resulting phylogenetic hypotheses were used to test the monophyly and relationships of Kupicha's *Lathyrus* sections and the validity of previously proposed classification systems for the genus *Lathyrus,* as well as to answer evolutionary questions presented by these classification schemes.

MATERIALS AND METHODS

Sampling—Forty-two of the 161 currently recognized *Lathyrus* species were included and 12 of Kupicha's (1983) 13 sections were represented (Table 2). Material from *L. saxatilis* (Vent.) Vis., which comprises the monotypic section *Viciopsis,* was not available. For all sections with multiple species, more than one species was examined except for section *Lathyrostylis* where seeds of only one species would germinate. *Lathyrus japonicus* from section *Orobus* is a variable species (Bässler, 1973), which was represented by nine accessions to assess intraspecific variation. Two *Vicia* species, *V. cracca* and *V. pisiformis,*

were included as outgroups (Table 2). Most leaf materials used in this study originated from seeds obtained from botanical gardens (Table 2); these were then grown and maintained in a greenhouse at Oregon State University. Three North American species (*L. nevadensis, L. littoralis,* and *L. polyphyllus*) and two accessions of *L. japonicus* were collected in native habitats (Table 2). Vouchers are deposited at AAU and OSC (Table 2).

Outgroup choice—*Lathyrus* is one of five closely related genera constituting the tribe Vicieae along with *Vicia, Lens* J.Mill., *Pisum* L., and *Vavilovia* Al. Fed. (Kupicha, 1977, 1981). *Vicia* with 163 species is about the size of *Lathyrus*, whereas *Lens, Pisum,* and *Vavilovia* all are small genera comprising two, four, and one species, respectively (Ben-Ze'ev and Zohary, 1973; Ladizinsky et al., 1984; Hoey et al., 1996). Vicieae is monophyletic and is part of the clade of legume tribes lacking one copy of the cpDNA inverted repeats and probably developed from a herbaceous group belonging to Galegeae (Kupicha, 1977, 1981; Polhill, 1981; Sanderson and Liston, 1995; Endo and Ohashi, 1997). Vicieae species are characterized by hypogeal germination, an unusual stele type in which the lateral leaf traces are present as cortical bundles in the internode below the insertion of the leaf, presence of tendrils, a style perpendicular to the ovary, stylar hairs used as secondary pollen presenters, and the diploid chromosome number $2n = 14$ (Kupicha, 1975, 1977; Gunn and Kluve, 1976). The five genera belonging to Vicieae are often delimited on the basis of a combination of two or three characters, such as type of leaf vernation (conduplicate vs. supervolute), form of the end of the staminal tube (truncate vs. oblique), shape of the style (flat vs. terete and folded vs. nonfolded), and style indumentum distribution (hairs on the adaxial side vs. hairs on the abaxial side or all around the style). Chemotaxonomic studies have successfully found chemical compounds discriminating genera within Vicieae. *Lathyrus* and *Pisum* produce the phytoalexin pisatin, whereas *Vicia* and *Lens* produce another phytoalexin, wyerone (Robeson and Harborne, 1980). The free amino acid canavanine is present in some *Vicia* species (Bell, Lackey, and Polhill, 1978) and lathyrine, another free amino acid, has been found in *Lathyrus* species only (Bell, 1962, 1966). *Vicia* and *Lathyrus* share several plesiomorphic or parallel evolved character states, such as unijugate leaflets, species without tendrils, and a reduced number of flowers in the annual species (Kupicha, 1976, 1983), and they are distinguished by ptyxis type, form of staminal tube, form of style, and type of phytoalexin and free amino acids but are probably morphologically best separated by the stylar pubescence. All *Lathyrus* species have adaxial stylar hairs, while *Vicia* species have either abaxial stylar hairs or hairs all around the style. In conclusion, *Vicia* and *Lathyrus* are well separated but closely related genera, which makes *Vicia* a suitable outgroup.

DNA isolation—Total genomic DNA was isolated from leaflets (stipules or phyllodes for plants without leaflets) using cetyltrimethylammonium bromide (CTAB) following the protocol of Doyle and Doyle (1987). Leaf material was taken from one individual representing the species except for *L. japonicus* where one individual from each of nine accessions was included.

PCR amplifications—Two discrete chloroplast regions were amplified using the polymerase chain reaction (PCR). One region, *rpo*C, consists of the *rpo*C1 (RNA polymerase C1) gene, its intron, part of the *rpo*C2 gene (RNA polymerase C2), and their intergenic spacer (Fig. 2). The other region, IR⁻, is positioned where one copy of the cpDNA inverted repeats is missing and IR⁻ spans the genes, *psb*A (photo system II D-I protein), *trn*H-GUG (histidine-GUG transfer RNA), part of *ndh*F (NADH dehydrogenase subunit 6), and their intergenic spacers (Fig. 2). The two oligonucleotide primers used to amplify the 4100-bp *rpo*C were: 5′-AAG CGG AAT TTG TGC TTG TG-3′ (*rpo*C1–195) and 5′-TAG ACA TCG GTA CTC CAG TGC-3′ (*rpo*C2–1364R; Liston, 1992). IR⁻ (3100 bp) was amplified using the primers: 5′-GAC TGC

TABLE 2. Collections of *Lathyrus* and *Vicia* species examined for *rpo*C and IR⁻ restriction site variation.

Genus and section[a]	Species	Seed or leaf source, location, and voucher[b]
Lathyrus L.		
Aphaca (J.Mill.) Dumort (2)	*L. aphaca* L.	Gatersleben LAT 141/76; Asmussen 1994-1
Clymenum (J.Mill.) DC. ex Ser. (3)	*L. clymenum* L.	Gatersleben LAT 113/75; Asmussen 1994-2
	L. gloeospermus Warb. & Eig	Southampton 868074; Syria; Asmussen 1994-3
	L. ochrus (L.) DC.	Gatersleben LAT 329/73; Asmussen 1994-4
Lathyrostylis (Griseb.) Bässler (20)	*L. digitatus* (M.Bieb.) Fiori	Gatersleben LAT 8/87; Crimea; Asmussen 1994-5
Lathyrus (33)	*L. amphicarpos* L.	Gatersleben LAT 139/84; Portugal; Asmussen 1994-6
	L. annuus L.	Gatersleben LAT 152/87; Israel; Asmussen 1994-7
	L. cicera L.	Gatersleben LAT 202/73; Asmussen 1994-8
	L. cirrhosus Ser.	Liège 4508; Asmussen 1994-9
	L. gorgoni Parl.	Gatersleben LAT 101/89; Greece, Crete; Asmussen 1994-10
	L. hierosolymitanus Boiss.	Gatersleben LAT 142/84; Asmussen 1994-11
	L. hirsutus L.	Gatersleben LAT 144/75; Asmussen 1994-12
	L. latifolius L.	Gatersleben LAT 26/88; Italy; Asmussen 1994-13
	L. odoratus L.	Liston; cult., Corvallis, OR, USA; Asmussen 1994-14
	L. sativus L.	Gatersleben LAT 4015/85; Asmussen 1994-15
	L. sylvestris L.	Gatersleben LAT 4/87; Asmussen 1994-16
	L. tingitanus L.	Gatersleben LAT 146/84; Asmussen 1994-17
	L. tuberosus L.	Gatersleben LAT 11/90; Asmussen 1994-18
Linearicarpus Kupicha (7)	*L. angulatus* L.	Gatersleben LAT 151/87; Portugal; Asmussen 1994-19
	L. sphaericus Retz.	Gatersleben LAT 134/75; Asmussen 1994-20
Neurolobus Bässler (1)	*L. neurolobus* Boiss. & Heldr.	Gatersleben LAT 19/82; Asmussen 1994-21
Nissolia (J.Mill.) Dumort. (1)	*L. nissolia* L.	Gatersleben LAT 136/75; Asmussen 1994-22
Notolathyrus Kupicha (23)	*L. magellanicus* Lam.	Whatson et al. 9140, Kew; Chile; Asmussen 1994-23
	L. nervosus Lam.	Montevideo 7014; Uruguay; Asmussen 1994-24
	L. paranensis Burkart	Montevideo 2717; Uruguay; Asmussen 1994-52
	L. pubescens Hook. & Arn.	Montevideo 7072; Uruguay; Asmussen 1994-25
Orobastrum Boiss. (1)	*L. setifolius* L.	Genova; Asmussen 1994-26
Orobon Tamamsch. (1)	*L. roseus* Steven	Göttingen 1014; Armenian; Asmussen 1994-27
Orobus (L.) Godr. (56)	*L. davidii* Hance	Gatersleben LAT 21/82; Asmussen 1994-28
	L. delnorticus C.L.Hitchc.	Broich 642; Asmussen 1994-29
	L. graminifolius (S.Watson) T.G.White	Arizona 92-0239; Pima Co., AZ, USA; Asmussen 1994-30
	L. japonicus Willd.	Abojohka, Norway; Asmussen 1994-31
	L. japonicus Willd.	Ajstrup, Denmark; Asmussen 1994-32
	L. japonicus Willd.	Berkeley 61.1389; Asmussen 1994-33
	L. japonicus Willd.	Copenhagen 1562 S1979-0987; Asmussen 1994-34
	L. japonicus Willd.	Hokkaido 40; Hokkaido, Japan; Asmussen 1994-35
	L. japonicus Willd.	Holden; Lake Co., OH, USA; Asmussen 1994-36
	L. japonicus Willd.	Klim, Denmark; Asmussen 1994-37
	L. japonicus Willd.	Osnabrück; Vaesternorrland, Sweden; Asmussen 1994-38
	L. japonicus Willd.	Uggerby, Denmark; Asmussen 1994-39
	L. jepsonii Greene	Broich 1278; USA; Asmussen 1994-40
	L. nevadensis S.Watson[c]	Josephine Co., OR, USA; Asmussen, Wheeler, and Wheeler 1994-41
	L. littoralis (Nutt.) Endl.[c]	Lincoln Co., OR, USA; Asmussen, Wheeler, and Wheeler 1994-42
	L. niger (L.) Bernh.	Gatersleben LAT 6/86; Hungary; Asmussen 1994-43
	L. palustris L.	Nantes 662; Asmussen 1994-44
	L. polyphyllus Nutt.[c]	Benton Co., OR, USA; Asmussen, Wheeler, and Wheeler 1994-45
	L. sulphureus Brewer	Broich 1131; Asmussen 1994-46
Pratensis Bässler (6)	*L. laxiflorus* (Desf.) Kuntze	Aarhus; Asmussen 1994-47
	L. pratensis L.	Aarhus; Asmussen 1994-48
Vicia L.		
Cracca Dumort.	*V. cracca* L.	Copenhagen 1764 44; Denmark, Skudelev; Asmussen 1994-49
Vicilla (Schur) Asch. & Graebn.	*V. pisiformis* L.	Halle; Sachsen-Anhalt; Asmussen 1994-51

[a] Number of species in the section according to Kupicha (1983) is given in parentheses.
[b] Name of institution or person from which seeds or leaf material were obtained followed by collection location, if known, and voucher number.
[c] *Lathyrus nevadensis*, *L. littoralis*, and *L. polyphyllus* were collected in natural habitats.

AAT TTT AGA GAG ACG CG-3′ (*psb*A-3) and 5′-AGG GAG AAG TAC ATA CCA ATG G-3′ (*ndh*F-731R; Liston, 1995). PCR amplification of these cpDNA regions followed the procedure of Arnold, Buckner, and Robinson (1991) with minor modifications (Liston, 1992; Liston and Wheeler, 1994). Amplifications were carried out using an MJ Research thermal cycler (Watertown, Massachusetts) programmed for 1 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C, with a final 7 min at 72°C. PCR products were separated on 0.8% agarose gels with a marker (*Hin*dIII–*Eco*RI digested lambda bacteriophage DNA) to determine the sizes and quantitative amounts of the products.

Primer design —The *rpo*C sequence could not be amplified in species of section *Orobus,* probably due to insertions or structural rearrange-
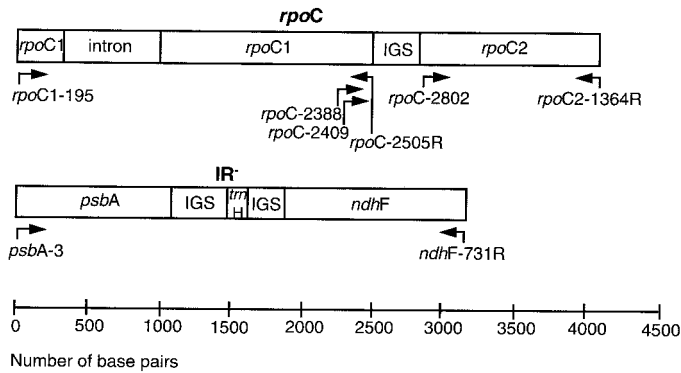
Fig. 2. The two cpDNA regions analyzed, *rpo*C and IR⁻, and the relative size of their genes and spacers. Name and position of the primers used to amplify these sequences are given. The primers *rpo*C1–195, *rpo*C2–1364R, *psb*A-3, and *ndh*F-731R are published in Liston (1992, 1995). The four internal primers of *rpo*C (*rpo*C-2388, *rpo*C-2409, *rpo*C-2505, and *rpo*C-2802) were designed for this study.

ments of the chloroplast genes. Two internal primers were therefore designed, with the aid of the computer program PRIMER (Lincoln, Daly, and Lander, 1991), in order to amplify *rpo*C as two parts: 5′-TAT GAC CAA CAG TGG TTC G-3′ (*rpo*C-2505R) and 5′-CCA TGA AAC GAC TTA TTA GT/CA GAT TAA T-3′ (*rpo*C-2802; Fig. 2). To assure primer specificity they were designed to include a conserved restriction site (universally present in other *Lathyrus* species) at the 3′ end. For the reverse primer *rpo*C-2505R it was a *Bst*BI site and for the forward primer *rpo*C-2802 an *Ase*I site. The primer *rpo*C-2505R was paired with primer *rpo*C1–195, and primer *rpo*C-2802 was paired with primer *rpo*C2–1364R. Using this approach, data from *rpo*C1 and *rpo*C2 were obtained for species belonging to section *Orobus*. Two attempts were made to include the intergenic spacer between *rpo*C1 and *rpo*C2 by designing forward primers at the 3′ end of the *rpo*C1 gene: 5′-GCT TCA AGA GAA ACT CCC/G AT-3′ (*rpo*C-2388) and 5′-GAA GTT CAC TAT GAA TCT TTN GGT ACC-3′ (*rpo*C-2409, *Kpn*I site; Fig. 2). The primer pair *rpo*C-2409 and *rpo*C2–1364R amplified *Lathyrus* species from other sections but not from section *Orobus*; primer pair *rpo*C-2388 and *rpo*C2–1364R amplified in tobacco but not in *Lathyrus* species.

The IR⁻ region would not amplify in *L. nissolia*. To determine whether both copies of the cpDNA inverted repeat regions were present in this species, the two primers *psb*A-3 and *trn*I-51R (5′-AGG TTC AAT TCC TAC TGG ATG C-3′; Liston, 1995) were used. This primer pair has routinely amplified a 3600-bp DNA fragment in legumes where both copies of the cpDNA inverted repeat are present but failed to amplify in *L. nissolia,* which indicates the presence of a structural rearrangement of the chloroplast genome prohibiting the test for presence or absence of one copy of the cpDNA inverted repeat.

Restriction endonuclease digests—The amplified *rpo*C products were digested with 31 restriction enzymes and the IR⁻ products with 27 restriction enzymes, each of which recognizes a unique 4-, 5-, or 6-bp sequence (Table 3). The many bands produced by the frequently cutting enzymes (*Alu*I, *Rsa*I, *Hin*fI, *Dpn*II, and *Bfa*I) in the *rpo*C region were difficult to interpret and thus the 2505-bp product resulting from amplification with the primers *rpo*C1–195 and *rpo*C1–2505R was used instead of the full-length *rpo*C (4100 bp). In this way only *rpo*C1 and its intron were assayed for the frequently cutting enzymes (Fig. 2). Fragments resulting from the digested PCR products were separated by electrophoresis in 1.4% agarose gels stained with ethidium bromide and photographed over UV light. PCR products digested with the frequently cutting enzymes were separated on 4% Metaphor agarose (FMC BioProducts, Rockland, Maryland) gels, which provide better resolution

of small and closely spaced fragments than conventional gel systems. The sizes of the digested PCR products were determined with reference to two markers. For the 1.4% agarose gels, a *Hin*dIII-*Eco*RI digested lambda bacteriophage DNA marker and a 100-bp marker (Life Technologies, Gaithersburg, Maryland) were used, while a 100-bp ladder and a pBR322-DNA-*Msp*I marker were used for the Metaphor agarose gels. In order to map the linear order of restriction sites in the two cpDNA regions and thus confirm homology of the sites, double digests were performed and interpreted with the aid of (1) restriction site data from *Astragalus* and related genera (Liston, 1992; Liston and Wheeler, 1994) and (2) sequence data from tobacco and rice (Shinozaki et al., 1986; Hiratsuka et al., 1989). Some restriction enzymes that recognize 4 and 6 bp, respectively, produce nonindependent restriction site characters because the recognition site of the four cutter enzyme is included in the recognition site of the six cutter enzyme, e.g., *Dpn*II (recognizing GATC) and *Bam*HI (recognizing GGATCC). These potentially redundant sites were all checked (for *rpo*C it was: *Rsa*I and *Kpn*I, *Alu*I and *Hin*dIII, *Bfa*I and *Xba*I, and *Dpn*II and *Bam*HI; and for IR⁻ it was *Dpn*II and *Bam*HI). For most of these corresponding sites the four cutter enzyme sites were invariant and not included as characters in the matrix, leaving the six cutter enzyme sites as nonredundant characters to be included. Two autapomorphic *Hin*dIII sites for *rpo*C were excluded because the corresponding *Alu*I recognition sites could be found. Length variations could be located within a few hundred base pairs by identifying homologous restriction sites among species with and without these mutations and by comparing the results from all restriction enzymes.

Data analyses—Nucleotide divergence was estimated by the maximum likelihood method of Nei and Tajima (1983) using the program SDE: Sequence Divergence Estimator version 1.2 (Wolfe and Wolfe, 1993). This program assumes that all sites are six 6-bp cutters, so it underestimates the value for restriction enzymes recognizing 4- and 5-bp sequences. Variable restriction sites were coded as present or absent in the parsimony analyses. Question marks represent sites in the DNA fragments that could not be amplified and are truly missing data (Nixon and Davis, 1991); they were coded as uncertainties. Structural rearrangements and length variations were not included as characters in the analyses. Unless otherwise specified, cladistic analyses were performed using PAUP version 3.1.1 (Swofford, 1993). To verify the consequence of having included missing data in the analyses, *L. nissolia, L. aphaca,* and the 12 species of section *Orobus* were removed sequentially as were the eight characters that had missing data for section *Orobus* members, and the data were reanalyzed (as in Bruneau and Doyle, 1993). In all analyses one of the two *Vicia* taxa included, *V. cracca,* was chosen as outgroup in the "define outgroup" option of PAUP. Because of the large size of the data matrix, the heuristic search algorithms were used. The MULPARS option and a heuristic search with simple stepwise addition and tree bisection reconnection (TBR) branch swapping were used. Consensus trees were constructed using the strict consensus option of PAUP. In addition to PAUP analyses, a Hennig86 version 1.5 (Farris, 1988) analysis of combined *rpo*C and IR⁻ data was performed with the approximate options mh* and bb*. The combined data set was also analyzed using Nona version 2.1 (Goloboff, 1994), with 1000 iterations of tree-construction using random taxon-entry sequences, with TBR swapping on up to 20 trees per iteration; the commands hold/20 and mult*1000 were used, with the option poly = (unsupported dichotomies collapsed), followed by additional TBR swapping to completion. This analysis was run twice, once with the default option amb- (ambiguously supported clades collapsed) and once with the option amb = (ambiguously supported clades resolved). Character-state weighting was implemented using the step-matrix options of PAUP. Implementation followed recommendations in Albert, Mishler, and Chase (1992) and Wendel and Albert (1992); costs of site gains over site losses were 1.8:1, 1.5:1, 1.3:1, and 1.1:1. The robustness of the clades was inferred by a bootstrap analysis of 100 replicates performed with TBR swapping and

TABLE 3. Restriction enzymes used to cut the two cpDNA regions, *rpo*C and IR⁻, and the sequences they recognize.

Enzyme	sequence	*rpo*C[a] No. sites[b]	Informative sites	Autapomorphies	IR⁻ No. sites[b]	Informative sites	Autapomorphies
*Alu*I	5′ … AGCT … 3′	8 (3)	3	2			
*Ase*I	5′ … ATTAAT … 3′	8 (3)	3	2	5	3	2
*Bam*HI	5′ … GGATCC … 3′	2 (1)	0	1	3 (1)	2	0
*Bcl*I	5′ … TGATCA … 3′				2 (1)	0	1
*Bfa*I	5′ … CTAG … 3′	6 (4)	1	1	12 (4)	1	7
*Bsa*JI	5′ … CCNNGG … 3′	3 (1)	0	2	5 (3)	0	2
*Bsm*AI	5′ … GTCTCN … 3′	7 (2)	3	2	4 (1)	1	2
*Bsr*I	5′ … ACTGGN … 3′	13 (4)	1	8	3	1	2
*Bst*BI	5′ … TTCGAA … 3′	10 (6)	2	2	3 (1)	1	1
*Bst*UI	5′ … CGCG … 3′	5 (2)	1	2	4 (1)	0	3
*Bst*XI	5′ … CCANNNNNNTGG … 3′	4 (1)	1	2			
*Cla*I	5′ … ATCGAT … 3′	4 (1)	1	2			
*Dde*I	5′ … CTNAG … 3′	8 (1)	3	4	5 (1)	2	2
*Dpn*II	5′ … GATC … 3′	13 (5)	6	2	16 (6)	3	7
*Dra*I	5′ … TTTAAA … 3′	5	3	2	4 (1)	0	3
*Eco*RI	5′ … GAATTC … 3′	6	3	3	3 (1)	2	0
*Eco*RV	5′ … GATATC … 3′	5 (3)	1	1			
*Hae*III	5′ … GGCC … 3′	4	2	2	3 (1)	1	1
*Hha*I	5′ … GCGC … 3′	5 (1)	1	3	4 (2)	0	2
*Hind*III	5′ … AAGCTT … 3′	3	1	2	4 (1)	3	0
*Hinf*I	5′ … GANTC … 3′	13 (4)	7	2	11 (4)	6	1
*Kpn*I	5′ … GGTACC … 3′	2 (1)	0	1			
*Msp*I	5′ … CCGG … 3′	5 (2)	2	1	4	1	3
*Nde*I	5′ … CATATG … 3′	2	2	0			
*Nsi*I	5′ … ATGCAT … 3′	2 (1)	0	1	3 (1)	0	2
*Pst*I	5′ … CTGCAG … 3′				1	1	0
*Pvu*II	5′ … CAGCTG … 3′				3 (1)	1	1
*Rsa*I	5′ … GTAC … 3′	7 (4)	1	2	8 (3)	4	1
*Sau*96I	5′ … GGNCC … 3′	7 (2)	4	1	5 (3)	0	2
*Scr*FI	5′ … CCNGG … 3′	9 (3)	4	2	3	1	2
*Ssp*I	5′ … AATATT … 3′	5 (1)	2	2	8 (4)	3	1
*Taq*I	5′ … TCGA … 3′				14 (3)	5	6
*Xba*I	5′ … TCTAGA … 3′	1	0	1			
*Xho*I	5′ … CTCGAG … 3′	3	3	0			
*Xmn*I	5′ … GAANNNNTTC … 3′	8 (1)	3	4	6 (1)	3	2
Total		183 (57)	64	62	146 (45)	45	56

[a] For the frequent cutting enzymes *Alu*I, *Rsa*I, *Hinf*I, *Dpn*II, and *Bfa*I a 2505-bp product of the *rpo*C region was digested and not the full 4100-bp *rpo*C product.

[b] Number of restriction sites scored for each enzyme with number of monomorphic sites in parentheses.

200 trees saved in each replicate (Felsenstein, 1985; Sanderson, 1989; Hillis and Bull, 1993; Swofford et al., 1996).

RESULTS

Restriction site mapping—A total of 183 *rpo*C restriction sites were observed (Table 3; data matrices available from authors upon request). Fifty-seven sites were invariant, 62 were autapomorphies, and the remaining 64 were cladistically informative synapomorphies (Table 3). The linear order and approximate position of 124 *rpo*C restriction sites excluding all autapomorphic site gains were mapped (Fig. 3). The positions of seven potentially informative sites and one monomorphic site were ambiguous and they were excluded from the map. A total of 146 restriction sites were observed in the region where one copy of the cpDNA inverted repeat is missing (IR⁻), 45 of these sites were invariant, 56 were autapomorphies, and 45 were synapomorphies (Table 3). The linear order and approximate position of 92 sites were mapped excluding autapomorphic site gains (Fig. 4). The positions of six potentially informative sites were ambiguous and

they were excluded from the map. Autapomorphic site gains were not included in the maps because it was decided that the work involved would exceed the value of knowing the position of a site present in a single species. Data from the intergenic spacer between *rpo*C1 and *rpo*C2 are missing for all species belonging to section *Orobus*; IR⁻ data are missing for *Lathyrus nissolia* (see Methods). A total of 892 nucleotides were sampled within *rpo*C and 681 nucleotides within IR⁻. Percentage cpDNA sequence divergence, (substitution/nucleotide) × 100, calculated for each species pair ranged from 0 to 9.83%, mean and SD = 4.32 ± 1.67% (sequence divergence data available from authors upon request).

Structural rearrangements and length variations—In addition to base pair mutations, four length variations and two putative structural rearrangements were identified (Figs. 3–5). The *rpo*C region varied in length within section *Orobus* between bp 1030 and bp 1270. The polymorphism was in the coding region of *rpo*C1 and varied between an additional 20–375 bp among the *Orobus* spe-
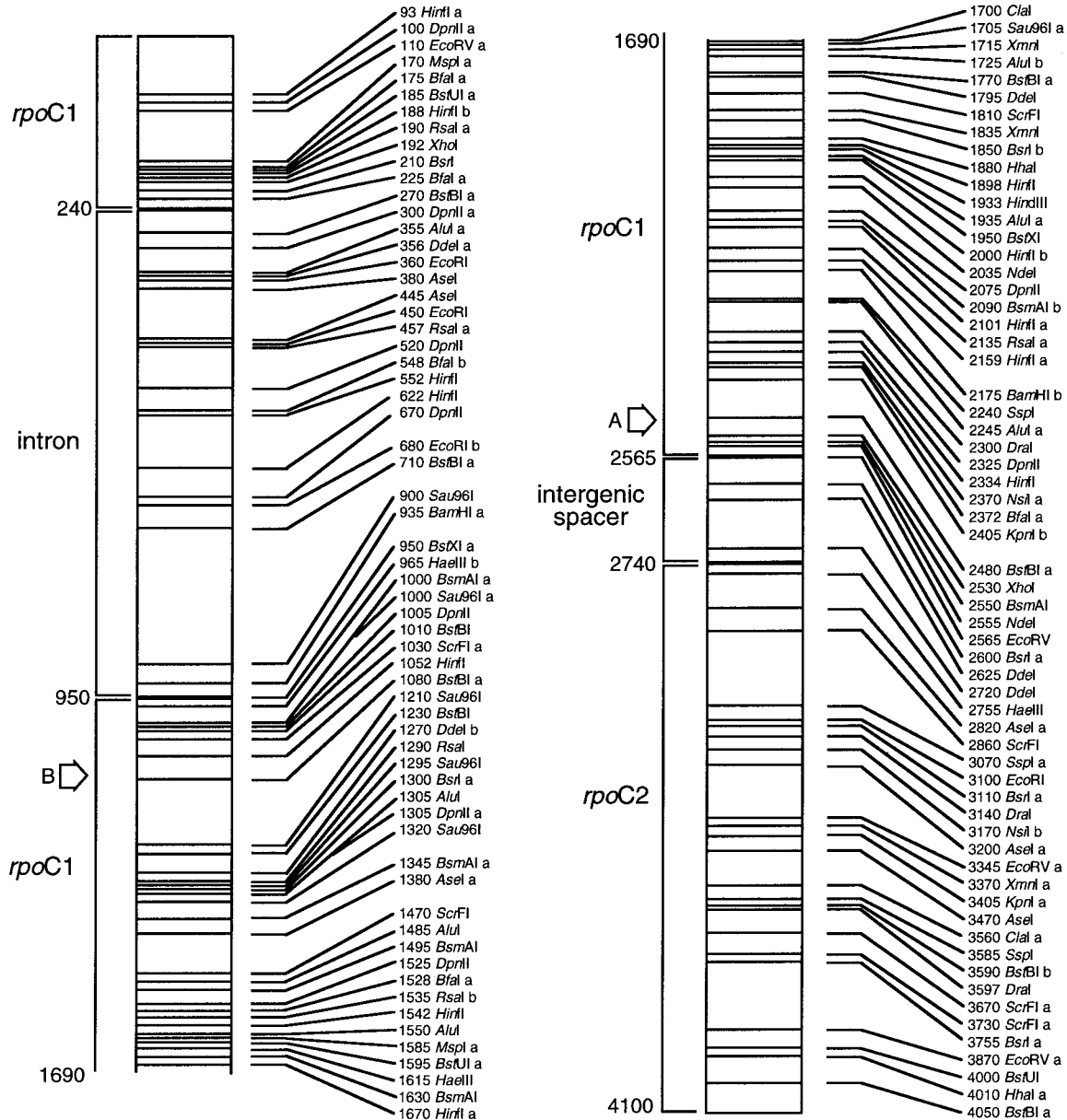
Fig. 3. The linear order and approximate position of the 124 mapped *rpo*C restriction sites. Autapomorphic site gains and ambiguous sites are excluded. The approximate position of a site is given as a base pair number starting with one at the beginning of the *rpo*C region; a indicates a monomorphic site and b an autapomorphic site loss. Structural rearrangements and length variations are marked by an arrow. The letter A indicates a putative structural rearrangement of the chloroplast that prevented amplification of *rpo*C and B represents an insertion of 20—375 bp between bps 1030 and 1270 in *rpo*C1.

cies. Three length differences occurred in the IR⁻ region. All *Lathyrus* species were ~100 bp shorter than the two *Vicia* species between bp 1000 and bp 1552 in the IR⁻ region. This mutation is either in the spacer between *psb*A and *trn*H-GUG or in *trn*H-GUG itself; the former is more likely because *trn*H-GUG is short (74 bp) and conserved. A clade of species belonging to section *Lathyrus* and *L. tingitanus* (section *Lathyrus*) were 100 bp shorter between bp 1569 and bp 1811 in the IR⁻ region (Fig. 5). This length mutation is either in the *trn*H-GUG gene or more likely in the spacer between *trn*H-GUG and *ndh*F. *Lathyrus tingitanus* was another 75 bp shorter between bps 889 and 1452. This mutation is either in *psb*A

or in the spacer between *psb*A and *trn*H-GUG. All taxa belonging to section *Orobus* apparently share an insertion or a structural rearrangement that prevents amplification of the *rpo*C region. IR⁻ could not be amplified in *Lathyrus nissolia*, probably due to rearrangements of chloroplast genes and spacers.

Intraspecific variation in Lathyrus japonicus—In *Lathyrus japonicus*, 3.16% of the *rpo*C sites were variable (three of 95 sites) and the number of variable IR⁻ sites were 3.26% (three of 92 sites). The variation was limited to three of the nine populations. Percentage cpDNA sequence divergence, calculated for each acces-
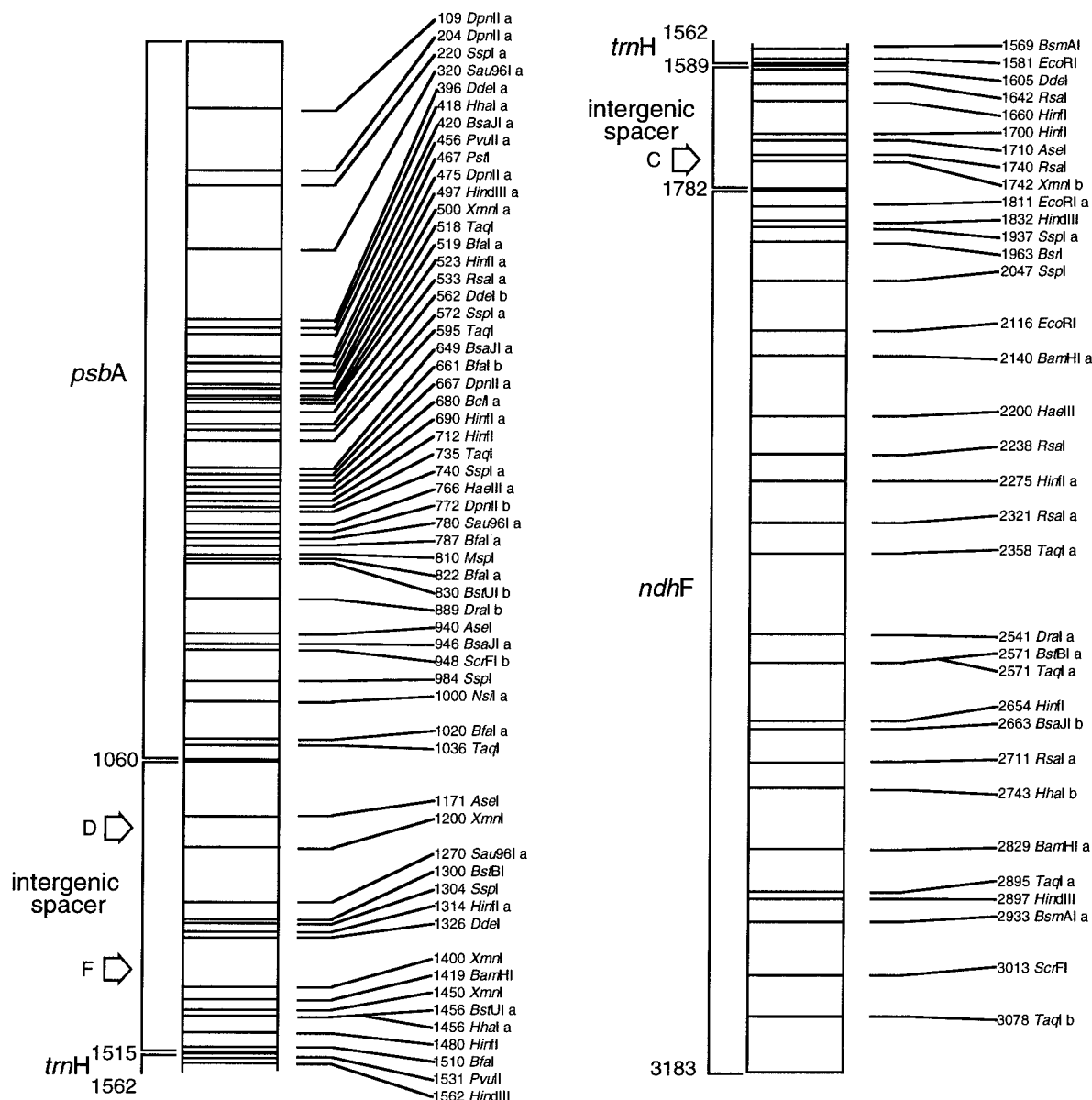
Fig. 4. The linear order and approximate position of the 92 mapped IR⁻ restriction sites. Autapomorphic site gains and ambiguous sites are excluded. The approximate position of a site is given as a base pair number starting with one at the beginning of the IR⁻ region; a indicates a monomorphic site and b an autapomorphic site loss. Structural rearrangements and length variations are marked by an arrow. The letter C is a 100-bp deletion in IR⁻ between bps 1569 and 1811, D represents a 75-bp deletion between bps 889 and 1452 in the IR⁻ region, and F a 100-bp deletion in the IR⁻ region between bps 1000 and 1500.

sion pair, (substitution/nucleotide) x 100, ranged from 0 to 0.23%.

Phylogenetic reconstructions—The result of the unweighted Wagner parsimony analysis of combined *rpo*C and IR⁻ data using PAUP resulted in 18 500 equally most parsimonious trees with lengths 283 (401 including autapomorphies), consistency indices of 0.385 (0.566 with autapomorphies), and retention indices of 0.725. The two Nona and the Hennig86 analyses resulted in 1200, 12 000, and >1400 trees, respectively, with the same tree lengths and strict consensus trees as the PAUP analysis (Fig. 5). Structural rearrangements and length variations were consistent with the strict consensus tree (Fig. 5).

The 1.3:1 and 1.1:1 character-state weighting analyses of the combined *rpo*C and IR⁻ data both resulted in 48 equally most parsimonious trees, each equivalent to a 283 step unweighted tree (401 including autapomorphies). These 48 trees showed only minor differences, all within the section *Orobus* clade, and the trees were subsets of the unweighted Wagner trees with the same tree lengths (Figs. 5, 6). The 1.5:1 and 1.8:1 character-state weighting resulted in 72 and 16 most parsimonious trees with unweighted tree lengths of 287 (405 including autapomorphies) and 293 (411 including autapomorphies) steps, respectively, which is four and ten steps longer than the most parsimonious trees identified in the unweighted analysis. All well-supported clades were the same in the
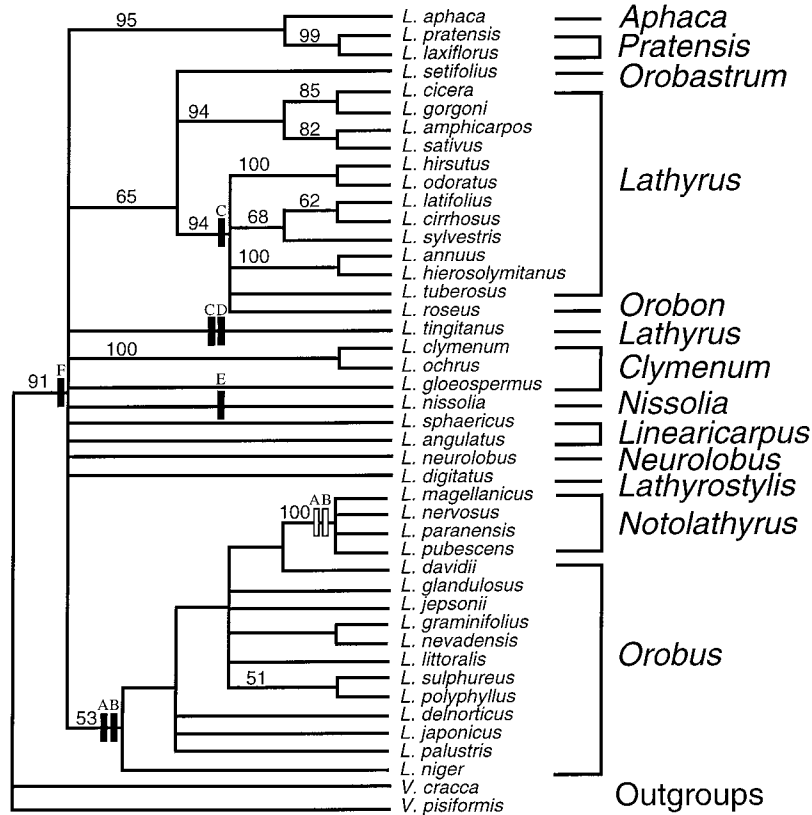
Fig. 5. Strict consensus tree of 18 500 equally most parsimonious trees resulting from Wagner parsimony analysis of combined *rpo*C and IR⁻ cpDNA restriction site data. The trees are 283 steps long and have consistency indices of 0.385 and retention indices of 0.725. Kupicha's (1983) sections are indicated at the right. The black rectangles represent indels or structural rearrangements: A is a putative structural rearrangement of the chloroplast that prevented amplification of *rpo*C, B represents an insertion of 20–375 bp between bps 1030 and 1270 in *rpo*C1, C is a 100-bp deletion in IR⁻ between bps 1569 and 1811, D represents a 75-bp deletion between bps 889 and 1452 in the IR⁻ region, E is a putative structural rearrangement preventing amplification of IR⁻, and F a 100-bp deletion in the IR⁻ region between bps 1000 and 1500. The white rectangles are reversals of the mutations represented by A and B. Bootstrap values (%) are given above the branches.

weighted analyses except for the section *Orobus* and *Notolathyrus* clade in the 1.8:1 weighted analysis, where section *Notolathyrus* and the smaller clades within section *Orobus* were arranged as a basal ladder instead of a monophyletic clade. The strict consensus tree of the 1.5:1 weighting differed from the 1.3:1 and 1.1:1 weighting by shifting the position of certain clades, e.g., the clades of sections *Pratensis, Aphaca, Clymenum* (except *L. gloeospermus*), *Nissolia,* and *L. neurolobus/L. tingitanus* were grouped with the section *Lathyrus* species in the 1.3:1 and 1.1:1 weighted analyses and grouped with the section *Orobus* clade in the 1.5:1 analysis.

The character-state weighting of restriction sites is thought to be theoretically preferable to Wagner parsimony analysis, in particular a 1.3:1 weighting, because it optimizes restriction site losses over parallel site gains (Albert, Mishler, and Chase, 1992; Wendel and Albert, 1992). Differences in results from the weighting schemes used were the number of trees, the tree lengths, and in particular the positions of *L. digitatus, L. angulatus, L. sphaericus,* and the clade of section *Orobus* and *Notolathyrus.* There were no conflicts between the strict consensus tree of the 1.3:1 weighted, 1.1:1 weighted, and the nonweighted data sets; discrepancies started when the higher weighting of 1.5:1 and 1.8:1 were applied. All of

the clades in the unweighted Wagner strict consensus topology are also present in strict consensus trees from the 1.5:1, 1.3:1, and 1.1:1 weighted trees, which makes all conclusions from the Wagner tree applicable to most weighted results.

The phylogenies resulting from all analyses positioned *Lathyrus* as monophyletic relative to the two *Vicia* species included. In the phylogeny derived from Wagner parsimony analysis of *rpo*C and IR⁻ data the *Lathyrus* clade was supported by three unique character changes and five homoplasious changes, in addition to a ~100-bp deletion in the IR⁻ sequence (Fig. 5). The more important features of the phylogeny derived from Wagner parsimony analysis of combined *rpo*C and IR⁻ data were: (1) section *Orobus* is paraphyletic with respect to section *Notolathyrus*; (2) the morphologically closely related *L. clymenum* and *L. ochrus* (both section *Clymenum*) were grouped together; (3) *L. gloeospermus* (also section *Clymenum*) was part of an unresolved clade in the unweighted analysis, but in the weighted phylogenies it was basal to all *Lathyrus* species; (4) all Kupicha's section *Lathyrus* members except *L. tingitanus* constitute a clade that also includes *L. setifolius* (from section *Orobastrum*) and *L. roseus* (from section *Orobon*); (5) *Lathyrus pratensis* and *L. laxiflorus,* both from section *Pratensis,* and *L. aphaca*
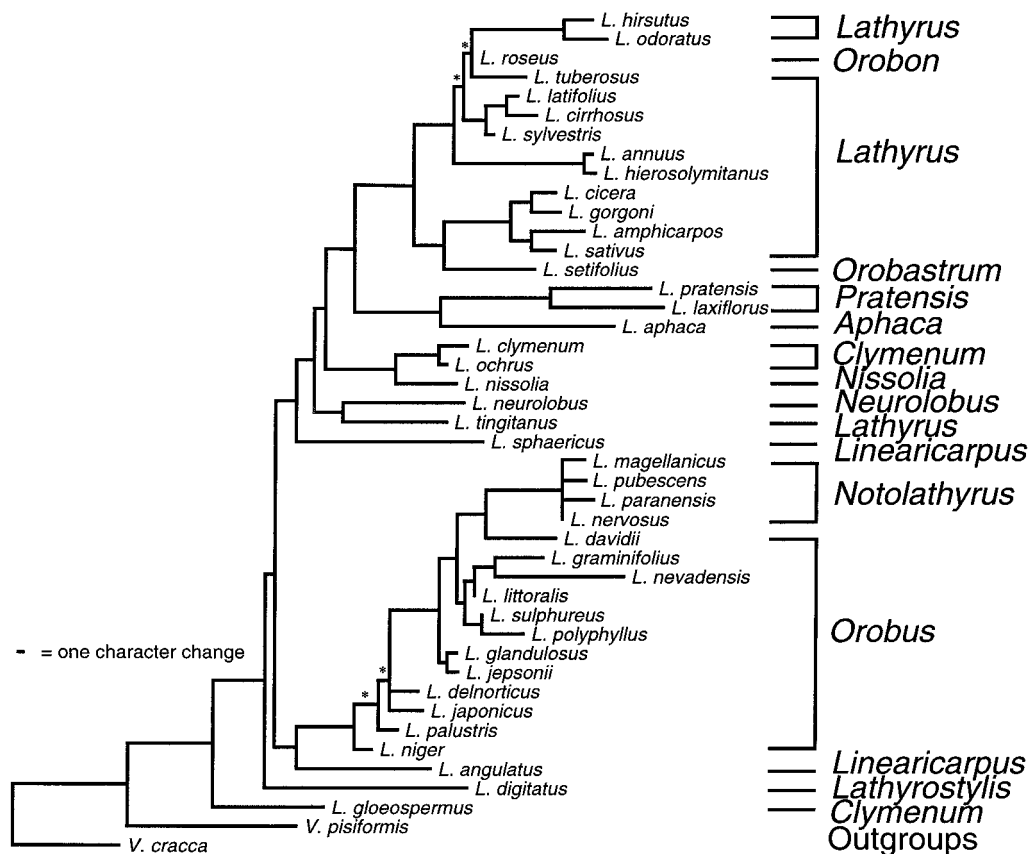
Fig. 6. One of the 18 500 Wagner trees that is also represented among the 48 trees resulting from the 1.3:1 and 1.1:1 character-state weighting analyses of combined *rpo*C and IR⁻ cpDNA restriction site data. The branch lengths represent relative number of character changes. Nodes that collapse in the strict consensus tree of the 48 trees resulting from the 1.3:1 and 1.1:1 character-state weighting analyses are marked by an asterisk. Kupicha's (1983) sections are indicated at the right.

from section *Aphaca* form a strongly supported clade that is characterized morphologically by a unique vascular pattern and a distinctive wing petal morphology. The monophyly of the clades above is supported by high bootstrap values (>90%) with the exceptions of section *Orobus* (53%) and section *Lathyrus* (65%).

The matrix of combined *rpo*C and IR⁻ data included 2% missing values, all within *L. aphaca, L. nissolia,* and the 12 species from section *Orobus*. When one of *L. aphaca, L. nissolia, L. glandulosus, L. jepsonii, L. niger, L. palustris,* and *L. polyphyllus* was excluded and data reanalyzed, the strict consensus trees were identical to the strict consensus tree resulting from analysis of the total data matrix. When one of *L. davidii, L. delnorticus, L. graminifolius, L. japonicus, L. littoralis, L. nevadensis,* and *L. sulphureus* was excluded and data reanalyzed, it resulted in minor changes within the section *Orobus* clade. Excluding *L. sulphureus* gave a less resolved *Orobus* clade, whereas sequentially leaving out the other seven species resulted in an additional 2–5 resolved nodes. Thus, missing data did not affect any major clades of the topology of the strict consensus tree, but the exclusion of particular species resulted in a considerable reduction in number of equally most parsimonious trees, e.g., a total of 1080 most parsimonious trees when leaving *L. graminifolius* or *L. nevadensis* out and 1440, 1800, and 2160 trees, respectively, when leaving *L. littoralis, L. delnor-*

ticus, or *L. japonicus* out. This result can be ascribed not only to the effect of missing data for these species but probably more to the fact that they are labile species with several most parsimonious positions for the data analyzed.

DISCUSSION

The sequence divergence values found in this study were relatively high (range = 0–9.83%, mean and SD = 4.32 ± 1.67%) compared to studies of restriction enzyme digestions of PCR products in other plant taxa (Liston, 1992; Rieseberg, Hanson, and Philbrick, 1992; Liston and Wheeler, 1994; Badenes and Parfitt, 1995; Tsumura et al., 1995, 1996; Wolfe et al., 1997). This suggests that *Lathyrus* has an elevated rate of cpDNA evolution compared to other examined genera. The PCR-RFLP method may not be as successful in resolving interspecific relationships whithin genera that have a lower rate of cpDNA evolution. The sequence divergence value of 0.23% found within *L. japonicus* (six variable sites in 187 sites surveyed) was between values reported from two other studies of intraspecific variation (Cipriani and Morgante, 1993; El Mousadik and Petit, 1996). The low intraspecific variation within the morphologically variable species *L. japonicus* justifies inclusion of data from just one representative of each species in the analyses.

Monophyly of Kupicha's sections, previous classification systems, and cpDNA phylogeny of Lathyrus—The strict consensus tree resulting from Wagner parsimony analysis of combined *rpo*C and IR⁻ cpDNA restriction site data suggests that: (1) several of Kupicha's *Lathyrus* sections may be combined in order to render monophyletic groups interpreted as sections; (2) the circumscriptions of some sections are in accordance with previously proposed classification schemes for the genus *Lathyrus* whereas other sections are rejected or redefined (Godron, 1848; Boissier, 1872; Bässler, 1966; Davis, 1970; Czefranova, 1971; Dogan, Kence, and Tigin, 1992; Fig. 1); and (3) there are no groupings that can be interpreted as the subgenera recognized in previous treatments of the genus *Lathyrus* (Bässler, 1966; Czefranova, 1971; Dogan, Kence, and Tigin, 1992). The positions of most sections relative to one another remain unresolved when interpreted according to the unweighted parsimony analysis (Fig. 5), but when the more resolved weighted analysis is considered the basal nodes are resolved and this gives an hypothesis for the relationships among sections (Fig. 6).

Sections Orobus and Notolathyrus—*Orobus* with 54 species is the largest and most diverse section in the genus *Lathyrus* (Bässler, 1966, 1973; Kupicha, 1983; Goyder, 1986). No diagnostic morphological characters exist for *Orobus*, but all species are perennial with multijugate leaves and many-flowered inflorescences. The phylogenetic hypothesis derived from molecular characters presented in this study suggests that Kupicha's section *Orobus* (12 of the 54 species were included) is monophyletic only when the South American section *Notolathyrus* (here represented by four of 24 species) is included (Fig. 5). Bässler (1966, 1973, 1981), Davis (1970), and Kupicha (1983) consider species of section *Orobus* basal to the genus *Lathyrus* and suggest that *Orobus* might be split into more sections, but few diagnostic characters for the new sections are available. Based on the taxa investigated in this study, the two sections should be combined until more data become available. Kupicha's section *Notolathyrus* is homogeneous and cpDNA data support *Notolathyrus* as a clade of closely related species. The position of the South American section *Notolathyrus* (including *L. pusillus* Elliott, which extends into North America) within the widespread section *Orobus,* to which all the North American *Lathyrus* species belong, is interesting because members of section *Notolathyrus* share morphological character states (e.g., narrowly elliptic leaflets, parallel-veined leaflets, spatulate style, divided stigma, septa between the seeds) with Turkish and Mediterranean species of section *Lathyrostylis, Clymenum, Linearicarpus,* and *Nissolia* (Simola, 1968a, 1986; Kupicha, 1983; Goyder, 1986). This predicted dispersal route of section *Notolathyrus* from North to South America follows the holarctic flora element theory on origin of Andean species, as well as the boreotropical hypothesis on biogeography when this theory is broadened to include temperate taxa (Cleef, 1979; van der Hammen and Cleef, 1986; Simpson and Todzia, 1990; Lavin and Luckow, 1993). The theory of South American species being derived from North American lineages is consistent with Kupicha (1983) who hypothesized that *Lathyrus* originated at high latitudes in the Old World, in the Cretaceous or early Tertiary. From this area original *Lathyrus* species with characteristics of section *Orobus* migrated to North America either through Greenland or from Asia through Alaska and then from North to South America where section *Notolathyrus* evolved. The results from cpDNA parsimony analyses contradict the hypothesis of Simola (1968a, 1986), who argue for an earlier evolution of *Lathyrus,* thereby making migration of *Lathyrus* species from southern Europe to South America possible. There are no fossil records of *Lathyrus* that can verify either of the two hypotheses.

Sections Aphaca and Pratensis—Morphologically and anatomically, section *Aphaca* (here represented by *L. aphaca*) and section *Pratensis* (here represented by *L. pratensis* and *L. laxiflorus*) share several putative synapomorphies such as wing-petals with an extension above the attachments, hastate stipules, and a unique pattern of nodal vascular anatomy where the petiole is supplied only by the median traces. Kupicha's reasons for keeping them separate are the annual habit of section *Aphaca* in addition to its large stipules and the lack of leaflets, and the uniformity of section *Pratensis,* which includes perennial species with unijugate leaves and elliptic, parallel-veined leaflets. The cpDNA data support a close relationship between sections *Aphaca* and *Pratensis. Lathyrus aphaca* was characterized by many autapomorphies, but it also shared several synapomorphic character states with *L. pratensis* and *L. laxiflorus* (Fig. 5). Since *L. aphaca* was not nested within section *Pratensis* and since both sections are well characterized molecularly as well as morphologically, the two sections will be kept apart, in contrast to the treatment of Dogan, Kence, and Tigin (1992), who include section *Pratensis* in section *Aphaca* (Fig. 1). Both sections are well studied, well understood, and universally accepted as closely related taxa (Brunsberg, 1977; Kupicha, 1983) and the interesting question is not whether they should be accepted as one or two sections but where the combined clade belongs in the infrageneric phylogeny of the genus *Lathyrus.*

Sections Lathyrus, Orobon, and Orobastrum—Kupicha's section *Lathyrus* contains 33 species including most of the *Lathyrus* species grown commercially, such as the ornamentals *L. odoratus* (sweet pea) and *L. latifolius,* and the food and forage plants *L. sativus* and *L. hirsutus.* Section *Lathyrus* formed a fairly well-supported clade in the cpDNA phylogeny when section *Orobon* and section *Orobastrum* were included. *Lathyrus tingitanus,* which morphologically belongs to Kupicha's section *Lathyrus,* was positioned at the basal polytomy in the strict consensus tree. This position is explained by the many homoplasious character states of *L. tingitanus,* which caused it to be variably placed among the most parsimonious set of trees. The ~100-bp deletion, which characterized a clade of section *Lathyrus* species, was also found in *L. tingitanus* and supported its traditional affiliation with these species (Fig. 5). A reanalysis of the data with *L. tingitanus* constrained to the clade of section *Lathyrus, Orobon,* and *Orobastrum* resulted in most parsimonious trees, which were only three steps longer than those resulting from the unconstrained analysis.

Section *Lathyrus* and section *Orobon* (monotypic) are closely related and share several potentially synapomorphic character states, including a twisted style, a broad standard without pouches, and large flowers (Kupicha, 1983). Two additional taxa, section *Lathyrostylis* and *L. sulphureus* (section *Orobus*), have members with a twisted style, which is probably not homologous with the twisted style found in section *Lathyrus* (Bässler, 1981; Kupicha, 1983). The annual and perennial species with a section *Lathyrus* type of twisted style have traditionally been divided into two or three sections. One is the monotypic section *Orobon* containing the perennial *L. roseus*, another is section *Lathyrus* (synonym *Eulathyrus*) including the remaining perennials and the annuals with an arcuate style (curved into an arch), and a third is the annual section *Cicercula* (Medic.) Godr. including species with a canaliculate style (with longitudinal channels) of which *L. hirsutus*, *L. annuus*, *L. hierosolymitanus*, *L. gorgoni*, *L. cicera*, *L. sativus*, and *L. amphicarpos* were included in this study (Godron, 1848; Boissier, 1872; Davis, 1970; Czefranova, 1971; Fig. 1). Kupicha (1983) found this division unnatural and combined sections *Cicercula* and *Lathyrus* but maintained section *Orobon*. Chloroplast DNA data included *L. roseus* (section *Orobon*) in section *Lathyrus*, an idea that was proposed earlier by Liston and Shmida (1987). Based on stomata index and style morphology of a new subspecies, *L. roseus* subsp. *hermonis*, Liston and Shmida (1987) concluded that the character states found in this subspecies were similar to character states found in section *Lathyrus* species, thereby weakening the sectional isolation of *Orobon*. Chloroplast DNA data showed a close relationship between *L. tuberosus* and *L. roseus* but nested them within section *Lathyrus* and thereby rejected the sectional isolation of *L. roseus* and *L. tuberosus* proposed by Dogan, Kence, and Tigin (1992; Fig. 5).

Section *Orobastrum* has traditionally been a group of species whose sectional affinity could not be determined. Kupicha (1983) reduced *Orobastrum* to a monotypic section comprising *L. setifolius* and described section *Linearicarpus* and a monotypic section *Viciopsis*, from species formerly included in section *Orobastrum* (Fig. 1; Table 1). Kupicha (1983) considered *L. setifolius* related to sections *Linearicarpus* and *Lathyrus* but absence of a twisted style prevented Kupicha from aligning *L. setifolius* with the morphologically similar annuals of section *Lathyrus*, although she mentions that Davis (Davis, 1958, cited in Kupicha, 1983) found specimens of *L. setifolius* with twisted styles. In the strict consensus tree *L. setifolius* is part of an unresolved node but in many of the most parsimonious trees it is grouped with the clade of *L. cicera*, *L. gorgoni*, *L. amphicarpos*, and *L. sativus*. It is important to include *L. setifolius* in future studies of this group of annuals from section *Lathyrus* to determine whether it really belongs to this clade or should be recognized as a distinct section.

In summary, the position of *L. tingitanus* outside the clade of its morphologically close relatives (sturdy annuals of section *Lathyrus*, e.g., *L. odoratus*) needs to be further investigated and if future studies confirm this position outside the clade of section *Lathyrus* members, *L. tingitanus* should be placed in its own monotypic section. The following two scenarios can be considered for sec-

tions *Orobon* and *Orobastrum*, and all Kupicha's section *Lathyrus* members except *L. tingitanus*. Retention of sections *Orobon* and *Orobastrum* has already been questioned and in order to define a monophyletic section *Lathyrus*, a large section characterized by a twisted style accommodating these sections could be erected. Alternatively, these sections could be redefined as three new sections: (1) *Cicercula*, including *L. cicera*, *L. sativus*, *L. amphicarpos*, and *L. gorgoni*, excluding *L. hirsutus*, *L. annuus*, and *L. hierosolymitanus*, which have previously been included here; (2) a monotypic section *Orobastrum* comprising *L. setifolius*; and (3) section *Lathyrus*, including all perennial species with a twisted style, in addition to *L. roseus* from section *Orobon* and several annual species (Fig. 5). A division in three sections would be supported by the ~100-bp deletion in the IR⁻ region, which characterized one clade within section *Lathyrus* (Fig. 5). These are the taxonomic options to be tested in future studies. Our preference for now is to accept one large section *Lathyrus*, knowing that further studies will reveal whether *L. setifolius* merits recognition as a distinct taxon or belongs with the annual section *Cicercula*. *Lathyrus roseus*, on the other hand, is a section *Lathyrus* species and should be placed there in future revisions of the genus.

Sections Clymenum and Nissolia—Monotypic section *Nissolia* is unique in having phyllodic leaves that lack a tendril and never produce leaflets. Kupicha's section *Clymenum*, also characterized by phyllodic leaves, contains three species, all of which are included in this study (*L. clymenum*, *L. ochrus*, and *L. gloeospermus*). Juvenile leaves of species from section *Clymenum* are phyllodic and leaflets are produced later during development. *Lathyrus clymenum* and *L. ochrus* are closely related and share potentially synapomorphic character states, such as very wide petiole wings, hollow finger-like pouches on the standard, and a spatulate style with a sterile fleshy mucro at the apex. *Lathyrus gloeospermus* differs by having numerous ribs on the fruit and by petiole wings that are less pronounced. The unweighted Wagner analysis grouped *L. clymenum* with *L. ochrus*, but the morphologically distinct *L. gloeospermus* was positioned at the unresolved basal node. *Lathyrus nissolia* is also positioned as part of the basal polytomy of 11 branches in the unweighted phylogeny (Fig. 5). Interestingly, *L. nissolia* occurs as sister to *L. clymenum* and *L. ochrus* in the weighted analyses, which might be an indication of its true position (Fig. 6). Traditionally the presence of phyllodic leaves in these two groups has been explained as independently evolved character states (Kupicha, 1983), but the weighted analyses suggest that phyllodic leaves may be homologous in sections *Clymenum* and *Nissolia*, but evolved in parallel in *L. gloeospermus*. The position of *L. gloeospermus* outside the rest of the *Lathyrus* clade in the weighted analysis may be due to many homoplasious character states or it may be a true link between *Lathyrus* and *Vicia* (Fig. 6). Dogan, Kence, and Tigin (1992) enlarged section *Clymenum* to include *L. setifolius* (section *Orobastrum*) and six annuals from Kupicha's section *Lathyrus* and expanded section *Nissolia* to include part of section *Linearicarpus* and section *Viciopsis* (*L. saxatilis*; Fig. 1). Chloroplast DNA data did

not agree with this alternative arrangement but confirmed the traditional circumscription of *Nissolia* and *Clymenum* except for *L. gloeospermus* from section *Clymenum*, the affiliation of which remains problematic (Fig. 5).

Sections Linearicarpus, Lathyrostylis, and Neurolobus—Kupicha's section *Linearicarpus* is defined as seven annual species with unwinged stems, a one-flowered inflorescence, and a narrowly linear fruit. These species were traditionally included in section *Orobastrum* (Kupicha, 1983). *Linearicarpus* was represented in the cpDNA phylogeny by the morphologically similar *L. angulatus* and *L. sphaericus*, both of which were part of the unresolved basal node (Fig. 5). *Lathyrus digitatus* is the only representative of the relatively uniform section *Lathyrostylis* (comprising 20 species) included in this study and its position was unresolved in the analysis of cpDNA data (Fig. 5). More thorough sampling of sections *Linearicarpus* and *Lathyrostylis* is needed before anything can be concluded about their monophyly and relationships to other *Lathyrus* species. *Lathyrus neurolobus,* from the monotypic section *Neurolobus*, is the last single member of the unresolved basal node. *Neurolobus* has a systematically isolated position (Bässler, 1966) and is regarded as a relict species (Kupicha, 1983). *Lathyrus neurolobus* is a morphologically distinct taxon because it is a perennial species that resembles annual *Lathyrus* species by being autogamous and having a less robust habit than other perennials in the genus, and it is unique in having small blue flowers, longitudinal stripes on the fruit, and two small elliptic leaflets. *Lathyrus neurolobus* is characterized by many autapomorphies and homoplasious character states (Fig. 6), but the position of section *Neurolobus* must await data from other molecular sources.

Conclusions—Infrageneric *Lathyrus* classification has varied remarkably during its history and classifications based on morphology are problematic due to homoplasy and lack of diagnostic characters, which to some extent also occurred in the classification based on cpDNA characters. The general trend in the cpDNA classification was to recognize fewer sections that encompassed more morphological variation. The cpDNA data suggested 6–8 sections, but left the classification of at least five species unresolved. One monotypic section, *Viciopsis,* was not sampled in this study. It is preferable to have DNA data from all species within a section before making a formal taxonomic redefinition, especially in a genus like *Lathyrus* where the homoplasy of morphological characters is high. Considering the potential for discrepancies between chloroplast ''gene trees'' and organismal ''species trees'' (Doyle, 1992), comparison to DNA phylogenies from nuclear genes is desirable. Several annual groups came out unresolved or differently than predicted from previous classifications, which are based on morphology. It is therefore important to include all annuals in future studies of the genus *Lathyrus*. The relationships of section *Lathyrostylis* to the rest of the genus *Lathyrus* needs to be studied by including more species. The intrasectional structure of *Orobus* including the South American section *Notolathyrus* could be studied rigorously by including more species, especially species related to *L. niger* and

L. davidii. Finally, biogeographical questions, such as what are the relationships among European, American, and Asian species, require further investigation.

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