Pl. Syst. Evol. 214: 49–64 (1999)

Plant Systematics and Evolution

The chloroplast genome arrangement of *Lobelia thuliniana* (*Lobeliaceae*): expansion of the inverted repeat in an ancestor of the *Campanulales*

ERIC B. KNOX and JEFFREY D. PALMER

Received May 14, 1997; in revised version August 26, 1997

Key words: *Campanulales*, *Campanulaceae*, *Cyphiaceae*, *Lobeliaceae*, *Sphenocleaceae*, *Lobelia thuliniana*. – Chloroplast DNA (cpDNA), inverted repeat (IR), genome rearrangements.

Abstract: A clone-bank of *Sac* I restriction fragments was constructed from the chloroplast DNA (cpDNA) of *Lobelia thuliniana* E. B. KNOX (*Lobeliaceae*). These cloned fragments and a set of 106 clones spanning the tobacco chloroplast genome were used as probes to determine the cpDNA restriction fragment arrangement for *Sac* I and six other restriction enzymes (*Bam*H I, *Eco*R V, *Hin*d III, *Nci* I, *Pst* I, and *Xho* I) and the chloroplast genome arrangement of *L. thuliniana* relative to tobacco, which has been fully sequenced and is collinear with the hypothesized ancestral genome arrangement of angiosperms. The results confirm and refine our previous understanding of the chloroplast genome arrangement in the large single-copy region (LSC) and reveal (1) a roughly 11 kilobase (kb) expansion of the inverted repeat (IR) into the small single-copy region (SSC) and (2) apparent sequence divergence of the DNA segment in *L. thuliniana* that corresponds to ORF1901 in tobacco. The expansion of the IR into the SSC is present in all other examined members of *Lobeliaceae*, *Cyphiaceae*, and *Campanulaceae*, which indicates that the IR expansion was an early event in the cpDNA evolution of the *Campanulales*. The IR expansion into the SSC was not present in *Sphenoclea*, which additionally supports exclusion of this genus from the *Campanulaceae*.

Rearrangements in the LSC of cpDNA among species of *Lobeliaceae* were previously shown to be phylogenetically useful traits that are concordant with the restriction-site data for these species (KNOX & al. 1993). Results from this previous Southern blot analysis indicated the presence of additional rearrangements involving the IR and SSC, but the heterologous lettuce and tobacco probes revealed a pattern sufficiently complex that the exact nature of the rearrangements could not be determined with the data available at that time. Specifically, (1) a probe covering 129.2–130.6 Tobacco Coordinate Units (TCU; a reference system in kilobase increments based on the completely sequenced tobacco chloroplast genome; see KNOX & al. 1993) failed to hybridize and suggested a partial deletion of ORF1901 (*ycf*1; see MAIER & al. 1995); (2) probes covering 119.5–129.2 TCU

revealed multiple shared fragments and suggested one or more rearrangements; and (3) a 2.7 kb segment of non-hybridizing DNA was detected between the genes normally found in the IR and SSC in non-rearranged genomes, and this non-hybridizing segment precluded any precise characterization of adjacent rearrangements.

In order to resolve this situation, we cloned the *Sac* I cpDNA fragments from *Lobelia thuliniana*, a giant lobelia from Iringa Region in southern Tanzania, which was included in a study of the giant lobelias in East Africa (KNOX & PALMER 1998). The use of homologous probes in a single and double-digest Southern blot analysis enabled us to determine the general cpDNA genome organization in this representative *Lobeliaceae* species, with restriction sites for seven restriction enzymes used as landmarks. We also probed these filters with 106 tobacco cpDNA subclones (see PALMER & al. 1994) which enabled us to infer more precisely the detailed genome arrangement of *L. thuliniana*. The *L. thuliniana* clones were also used to elucidate the SSC and IR arrangement in other species of *Lobeliaceae*, *Cyphiaceae*, *Campanulaceae*, and *Sphenocleaceae*.

Recombination within the IR results in two structural isomers of cpDNA that are present in equimolar concentrations (PALMER 1983), but recent cpDNA genome maps generally follow the convention used for tobacco (SHINOZAKI & al. 1986) of designating as IR_A the copy flanked by *trn*H and *psb*A in the LSC and ORF1901 and *rps*15 in the SSC and designating as IR_B the copy flanked by *rps*19 and *rpl*22 in the LSC and *ndh*F and *trn*L in the SSC. The junctions of these four regions have been similarly designated (SHINOZAKI & al. 1986) as J_{LA} (LSC/IR_A), J_{LB} (LSC/IR_B), J_{SA} (SSC/IR_A), and J_{SB} (SSC/IR_B). These labels are sufficient for a static representation of one of the two isomeric forms of a single cpDNA molecule, but are not sufficient for comparative descriptions of these structurally and evolutionarily dynamic molecules. The nucleotide sequence of the IR is maintained as two identical copies, presumably through a mechanism involving gene conversion and copy correction (GOULDING & al. 1996), so the differences between IR_A and IR_B are not differences within the IR per se, but differences with respect to the flanking regions of the LSC and SSC. Labels have apparently not been proposed for the two alternative combinations of flanking regions in the alternative structural isomer. The four junction labels can potentially be applied without reference to a particular structural isomer because they are designated with respect to the flanking regions of the LSC and SSC, but expansions and contractions of the IR can alter the gene content of the IR, LSC, and SSC, along with the positions of the junctions. The junctions themselves do not constitute landmarks, they are simply the points at which each region ends and the next region starts.

We therefore propose eight additional labels for comparative descriptions of the IR, with the tobacco cpDNA sequence (SHINOZAKI & al. 1986, OLMSTEAD & al. 1993) used as a reference system (Fig. 1). The standard structural isomer is defined as the orientation of the SSC relative to the LSC presented by SHINOZAKI & al. (1986). The alternative structural isomer is defined as the opposite orientation of the SSC relative to the LSC. IR_{LA} is defined as the portion of the IR adjacent to J_{LA}, with IR_{LB}, IR_{SA}, and IR_{SB} similarly defined with respect to J_{LB}, J_{SA}, and J_{SB}. IR_A is bounded by J_{LA} and J_{SA} and comprises IR_{LA} and IR_{SA}. Similarly, IR_B is bounded by J_{LB} and J_{SB} and comprises IR_{LB} and IR_{SB}. IR_C is defined as the IR

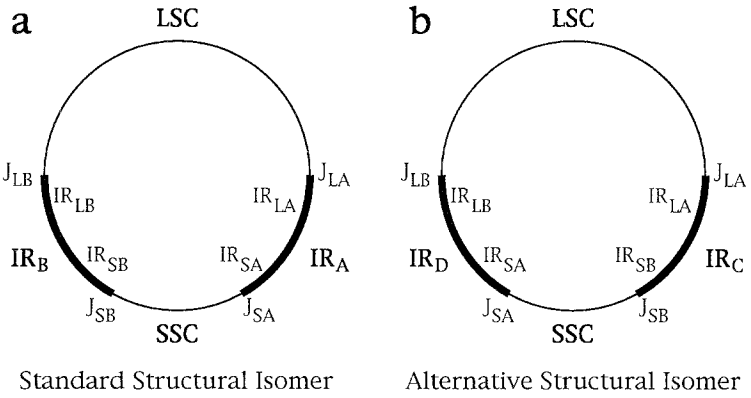

Fig. 1. Labels for the inverted repeats in the structural isomers of chloroplast DNA based on the convention used for tobacco (see text for definitions). *a* The standard structural isomer (see SHINOZAKI & al. 1986). *b* The alternative structural isomer

copy in the alternative structural isomer that is bounded by J_{LA} and J_{SB} and comprises IR_{LA} and IR_{SB}. IR_D is defined as the IR copy in the alternative structural isomer that is bounded by J_{LB} and J_{SA} and comprises IR_{LB} and IR_{SA}.

Materials and methods

Enriched cpDNA was extracted using the sucrose gradient method (PALMER 1986) from fresh leaves of a greenhouse specimen of *Lobelia thuliniana* that was grown at the Matthaei Botanic Garden, The University of Michigan, from field-collected seed (Table 1). An aliquot of this DNA was digested with *Sac* I and 'shotgun' cloned by ligating the fragments with Bluescript KS^+ plasmid, transforming competent *E. coli* cells, and screening on ampicillin/X-gal plates. Blue colonies were screened for the size of the inserted fragments and these were digested with frequently cutting enzymes (*Eco*R I or *Rsa* I) to differentiate between fragments of similar size. Of the five fragments not recovered using this method, three were successfully cloned using gel-isolated fragments, but two of the fragments (the 13.5 and 14.5 kb fragments; see Fig. 2) have not yet been cloned.

Enriched cpDNA aliquots of *Lobelia thuliniana* were digested with seven enzymes (*Bam*H I, *Eco*R V, *Hind* III, *Nci* I, *Pst* I, *Sac* I, and *Xho* I) individually and in *Sac* I double digests. The single and double digests were used in six replicate agarose gels and bidirectionally blotted to nylon filters (Zetabind™; AMF Cuno) to produce 12 replica filters according to the methods described by PALMER (1986) and PALMER & al. (1988a). Radioactive probes were made using random primers and the cloned *L. thuliniana* fragments (or gel-isolated fragments in the case of the 13.5 and 14.5 kb fragments) as templates. These probes were used to determine the identity and position of the *L. thuliniana* cpDNA *Sac* I fragments (requests regarding the availability of the *L. thuliniana* cpDNA clone bank should be directed to E. B. KNOX). The random priming method was also used to make probes from the tobacco subclones, which were used to map more precisely the restriction fragments of the six other enzymes used in the double-digests and to provide end-point estimates of the *Sac* I fragments and other structural features of the *L. thuliniana* cpDNA genome, such as the junctions of the IR, LSC, and SSC and the location of probing gaps relative to tobacco.

Table 1. Taxa surveyed for expansion of IR_{SA}. Accession numbers are used as voucher information for plants collected at Missouri Botanical Garden (MBG), the Pacific Tropical Botanical Garden (PTBG), the Royal Botanic Gardens, Kew (RBG), and the University of California Botanical Garden (UCBG). Vouchers are deposited at MICH unless otherwise indicated

Species	Source/voucher	IR expansion
Campanulaceae		
Codonopsis clematidea (SCHRENK) C. B. CLARKE	LAMMERS 8954 (F)	present
Cyphiaceae		
Cyphia decora THULIN	KNOX 2338	present
C. lasiandra DIELS	KNOX 2340	present
C. rogersii S. MOORE	DEPAMPHILIS s.n. (not vouchered)	present
Lobeliaceae		
Brighamia insignis A. GRAY	PTBG 90.5251	present
B. rockii H. ST. JOHN	PTBG 90.5503	present
Centropogon grandidentatus (SCHLDL.) ZAHLBR.	UCBG 81.0952	present
C. granulosus C. PRESL	MBG 89.4806	present
Clermontia fauriei H. LÉV.	KNOX 2376	present
Colensoa physaloides (A. CUNN.) HOOK. f.	KNOX 2373	present
Cyanea fissa (H. MANN) HILLEBR.	KNOX 2383	present
C. leptostegia A. GRAY	KNOX 2375	present
Heterotoma lobelioides ZUCC.	AYERS 633 (TEX)	present
Hippobroma longiflora (L.) G. DON	KNOX 2357	present
Isotoma anethifolia (SUMMERH.) E. WIMM.	KNOX 2367	present
I. axillaris LINDL.	KNOX 2368	present
I. fluviatilis (R. BR.) F. MÜLL. ex BENTH.	AYERS 978 (ASC)	present
Lobelia aberdarica R. E. FR. & T. C. E. FR.	KNOX 706	present
L. acrochilus (E. WIMM.) E. B. KNOX	KNOX 655	present
L. alata LABILL.	KNOX 2364	present
L. bambuseti R. E. FR. & T. C. E. FR.	KNOX 731	present
L. bequaertii DE WILD.	KNOX 244	present
L. blantyrensis E. WIMM.	KNOX 2325	present
L. boninensis KOIDZ.	RBG 194.85.01929	present
L. bridgesii HOOK. & ARN.	LAMMERS & BAEZA 6470 (F)	present
L. burttii E. A. BRUCE	KNOX 802	present
L. cardinalis L.	KNOX 883	present
L. chinensis LOUR.	UCBG 90.1141	present
L. columnaris HOOK. f.	HAMILTON s.n.	present
L. deckenii (ASCH.) HEMSL.	KNOX 52	present
L. dioica R. BR.	KNOX 2361	present
L. dunnii GREENE	AYERS 815 (ASC)	present
L. elongata SMALL	UCBG 82.2080	present
L. erinus L.	KNOX 2412	present
L. excelsa BONPL.	LAMMERS & al. 6393 (F)	present

(contd.)

Table 1 (continued)

Species	Source/voucher	IR expansion
L. fenestralis Cav.	Knox 2408	present
L. fervens Thunb.	Knox 602	present
L. giberroa Hemsl.	Knox 118	present
L. gloria-montis Rock	Smith 1175 (WIS)	present
L. gregoriana Baker f.	Knox 698	present
L. holstii Engl.	Knox 624	present
L. hypoleuca Hillebr.	Smith 1167 (WIS)	present
L. inflata L.	Knox 2401	present
L. kalmii L.	Knox 2409	present
L. kauaensis A. Heller	Knox 2381	present
L. laxiflora Humb., Bonpl. & Kunth	Ayers 394 (TEX)	present
L. longisepala Engl.	Knox 879	present
L. lukwangulensis Engl.	Knox 623	present
L. mildbraedii Engl.	Knox 873	present
L. minutula Engl.	Knox 550	present
L. morogoroensis E. B. Knox & Pócs	Pócs & Knox 88190/A	present
L. nicotianifolia Roem. & Schult.	RBG 394.85.04378	present
L. niihauensis H. St. John	PTBG 90.5315	present
L. organensis Gardner	RBG 195.85.02600	present
L. petiolata Hauman	Knox 134	present
L. polyphylla Hook. & Arn.	Lammers & al. 6331 (F)	present
L. portoricensis (Vatke) Urb.	Axelrod 2992	present
L. puberula Michx.	Knox 2404	present
L. purpurascens R. Br.	Knox 2365	present
L. ritabeaniana E. B. Knox	Knox 852	present
L. rhynchopetalum Hemsl.	Knox 650	present
L. sancta Thulin	Knox 610	present
L. × *speciosa* Sweet	Lammers 8708 (F)	present
L. spicata Lam.	Knox 2389	present
L. stenophylla Benth.	Knox 2362	present
L. stricklandiae Gilliland	Knox 881	present
L. stuhlmannii Stuhlmann	Knox 120	present
L. siphilitica L.	Knox 2410	present
L. telekii Schweinf.	Knox 689	present
L. tenuior·R. Br.	Lammers 8690 (F)	present
L. thuliniana E. B. Knox	Knox 876	present
L. trullifolia Hemsl.	Knox 2328	present
L. tupa L.	RBG 075.85.08148	present
L. wollastonii Baker f.	Knox 258	present
L. yuccoides Hillebr.	Knox 2379	present
Monopsis lutea (L.) Urb.	Ayers 610 (BH)	present
M. stellarioides (C. Presl) Urb.	Knox 1668	present
Pratia arenaria Hook. f.	Knox 2369	present
P. nummularia (Lam.) A. Br. & Asch.	Ayers 977 (ASC)	present
Rollandia crispa Gaudich.	Givnish 1038 (not vouchered)	present

(contd)

Table 1 (continued)

Species	Source/voucher	IR expansion
Sclerotheca jayorum J. RAYNAL	BERRY 4630 (PAP)	present
S. viridiflora CHEESEMAN	GAME 92/147 (not vouchered)	present
Trematolobelia kauaiensis (ROCK) SKOTTSB.	KNOX 2377	present
Sphenocleaceae		
Sphenoclea zeylanica GAERTN.	KNOX 2391	absent

Finally, the *Lobelia thuliniana* clone bank was used in conjunction with restriction-site mapping to survey the IR/SSC rearrangement in over 80 species of *Lobeliaceae* that represent *Brighamia*, *Centropogon*, *Clermontia*, *Colensoa*, *Cyanea*, *Heterotoma*, *Hippobroma*, *Isotoma*, *Lobelia*, *Monopsis*, *Pratia*, *Rollandia*, *Sclerotheca*, and *Trematolobelia* (Table 1), one species of *Codonopsis* (*Campanulaceae*), three species of *Cyphia* (*Cyphiaceae*), and one species of *Sphenoclea* (*Sphenocleaceae*).

Results

The cpDNA of *Lobelia thuliniana* is approximately 160.5 kb long (Fig. 2), with the LSC 80.2 kb long, the IR 36.2 kb long, and the SSC 7.9 kb long (Fig. 3). In comparison, the cpDNA of tobacco is 156.0 kb long, with the LSC 86.7 kb long, the IR 25.3 kb long, and the SSC 18.6 kb long (SHINOZAKI & al. 1986; see also

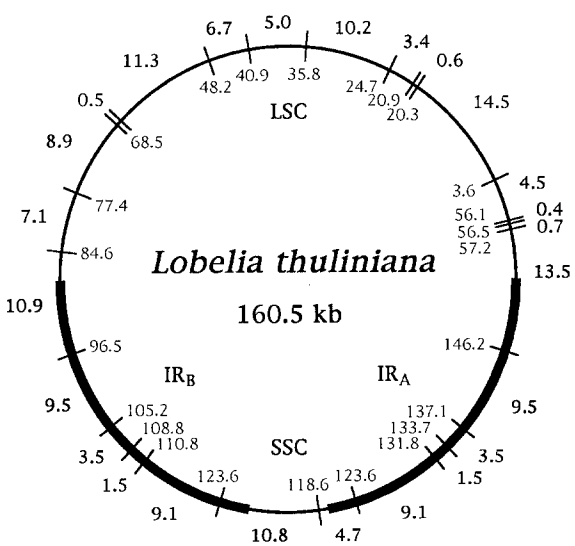

Fig. 2. Chloroplast genome arrangement of *Lobelia thuliniana*. Wide bands indicate the inverted repeat regions which separate the large and small single-copy regions. The outer numbers designate the size in kb of the *Sac* I fragments, and the inner numbers indicate the endpoints of these fragments in tobacco coordinate units (TCU; see KNOX & al. 1993)

OLMSTEAD & al. 1993). The significant differences from the hypothesized tobacco-like ancestral genome (PALMER & STEIN 1986) are (1) enlargement of the IR to include a roughly 9 kb segment (119.5–129.2 TCU) that was previously part of the SSC; (2) inclusion in the IR of a 2.7 kb segment that does not hybridize with the tobacco cpDNA but which may have been derived from ORF1901; (3) reduction of the region formally occupied by *acc*D from 2.8 kb to less than 1.7 kb; (4) small reductions in size at five locations in the genome (3.6–9.2, 27.5–32.2, 65.3–68.5, 80.4–82.5, and 109.7–110.8 TCU), presumably due to deletions of non-coding DNA; and (5) small increases at two locations (68.5 TCU and 82.5 TCU), presumably due to insertions of what is now non-coding DNA (Fig. 3). The probing results were consistent with all genes (except *acc*D and the entire ORF1901) being present and the same size as found in tobacco, with one exception (discussed below).

The lack of strong hybridization between the tobacco subclone probes and the *Lobelia thuliniana* cpDNA yielded five gaps in the tobacco subclone map. Gaps 1 and 2 (Fig. 3) are presumably non-coding DNA segments that remain after the loss of function of the *acc*D gene (included in tobacco subclones 45 and 46) and the disruption of the region by the two inferred inversions that resulted in the displaced inversion of the segment represented by tobacco subclones 40–44 (53.6–59.3 TCU; see KNOX & al. 1993). Gap 3 (68.5 TCU) is conservatively interpreted as resulting from a 0.5 kb insertion between ORF31 (*ycf*7; see MAIER & al. 1995) and *pet*G, but a size reduction in the adjacent region (65.3–68.5 TCU) raises the possibility that a small inversion occurred within the region covered by tobacco subclone 50 (67.1–68.5 TCU). It is not possible to differentiate between these two alternatives using Southern blot analysis, and this region needs to be sequenced before the exact position and orientation of *pet*G can be determined. Gap 4 (82.5 TCU) is presumably due to a small insertion because genes that flank this site (*inf*A and *rps*8) are separated by only 145 base pairs (bp) in tobacco. The genes contained on tobacco subclone 61 (80.7–82.5 TCU; *inf*A, *rpl*36, *rps*11, and most of *rpo*A) are tightly packed, comprising 1632 bp of the 1817 bp subclone, but the probing results (using restriction sites as landmarks) did not permit all four genes to fit within this region, and *rpo*A is arbitrarily drawn slightly smaller than the 1013 bp size of the gene in tobacco. Gap 5 is presumably due to sequence divergence (and possible loss of function) in ORF1901 because tobacco subclone 106 (129.2–130.6 TCU) did not hybridize with *L. thuliniana* cpDNA and subclone 105 (127.4–129.2 TCU) hybridized only weakly.

The expansion of the IR and concomitant reduction in the SSC of *Lobelia thuliniana* is apparently due to an expansion of IR_{SA}, accompanied by conversion of IR_{SB}, such that J_{SA} is now located at 119.5 TCU and J_{SB} is flanked by 119.5 TCU in the IR and 112.0 TCU in the SSC. The *ndh* genes A, E, G, H, and I, *rps*15, and the DNA sequence that corresponds to ORF1901 in tobacco are now included in the IR. This IR expansion was present in all species of *Lobeliaceae*, *Campanulaceae*, and *Cyphiaceae* surveyed (Table 1), but was not present in *Sphenoclea zeylanica*. These data suggest that expansion of the IR into the SSC constitutes an early phylogenetic marker for the evolution of *Lobeliaceae*/*Campanulaceae*/*Cyphiaceae* cpDNA from a tobacco-like ancestral genome arrangement.

Discussion

Rearrangements in cpDNA have served as useful phylogenetic markers in several diverse groups of plants (JANSEN & PALMER 1987; BRUNEAU & al. 1990; LAVIN & al. 1990; DOWNIE & PALMER 1992a, 1994; DOYLE & al. 1992, 1995; RAUBESON & JANSEN 1992a, b; STEIN & al. 1992; COSNER 1993; KNOX & al. 1993; KATAYAMA & OGIHARA 1996). The large number of rearrangements observed in *Lobeliaceae*, *Campanulaceae*, and *Cyphiaceae* cpDNA make the *Campanulales* a model system for studying the evolution of chloroplast genomic structure (COSNER 1993, KNOX

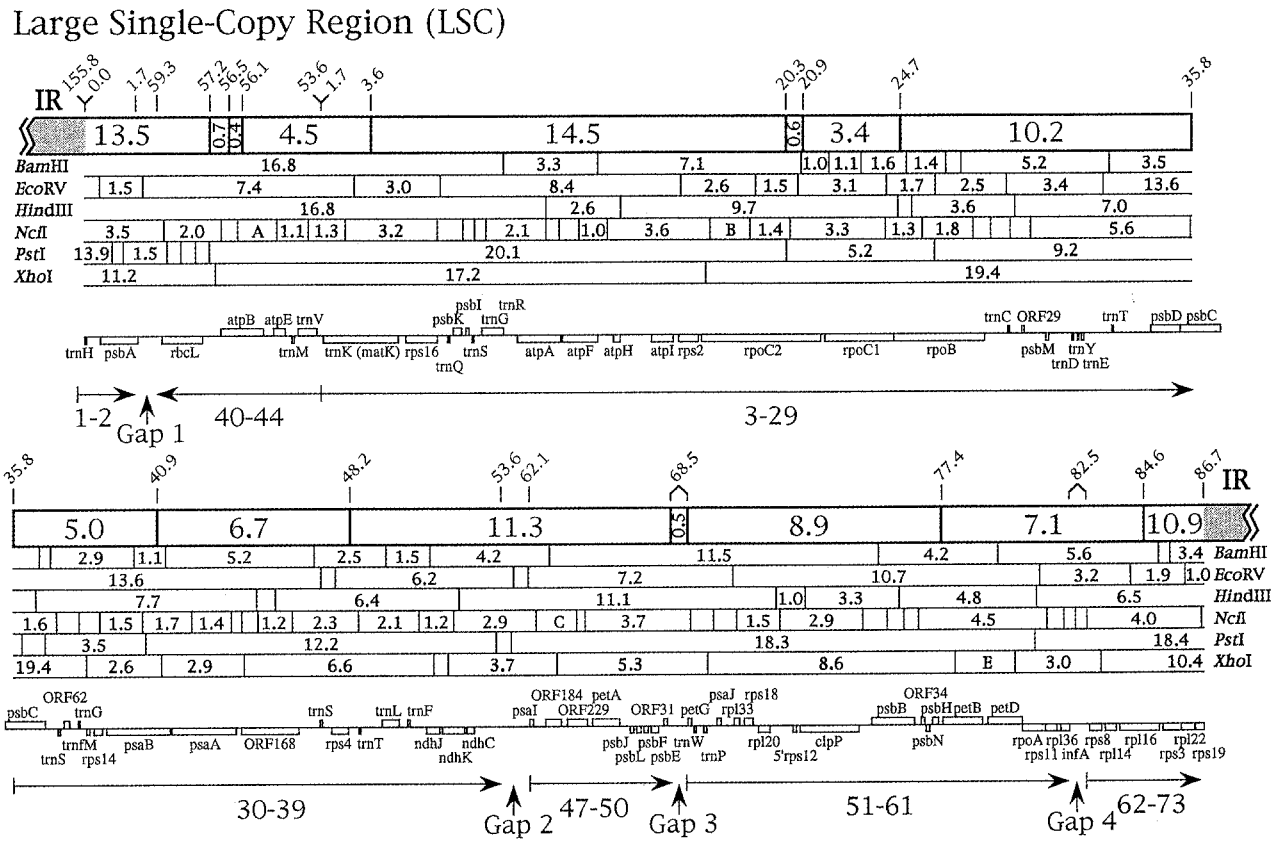

Fig. 3. A map of restriction sites, genes, and rearrangements in *Lobelia thuliniana* cpDNA relative to tobacco. The positions of *Sac* I fragment endpoints and other significant structural features of the *L. thuliniana* cpDNA genome are indicated in tobacco coordinate units (TCU; see KNOX & al. 1993). Maps of the restriction fragments for *Bam*H I, *Eco*R V, *Hin*d III, *Nci* I, *Pst* I, and *Xho* I are provided, with the sizes indicated for fragments 1.0 kb or larger. Upper-case letters indicate regions in which the linear sequence of small fragments could not be determined for *Nci* I (A = 0.9 and 0.3 kb; B = 1.0 and 0.4 kb; C = 0.8 and 0.6 kb; D = 0.7, 0.7, 0.7, 0.7, 0.4, and 0.4 kb), *Xho* I (E = 0.8, 0.7, 0.6, and 0.6 kb), and *Eco*R V (F = 1.4, 0.7, and 0.6 kb). The gene positions are interpreted from the position and orientation of the probing results using 106 tobacco subclones (PALMER & al. 1994). The gaps indicate segments of the *L. thuliniana* cpDNA that did not hybridize with any of the tobacco clones. See text regarding the size shown for *rpo*A

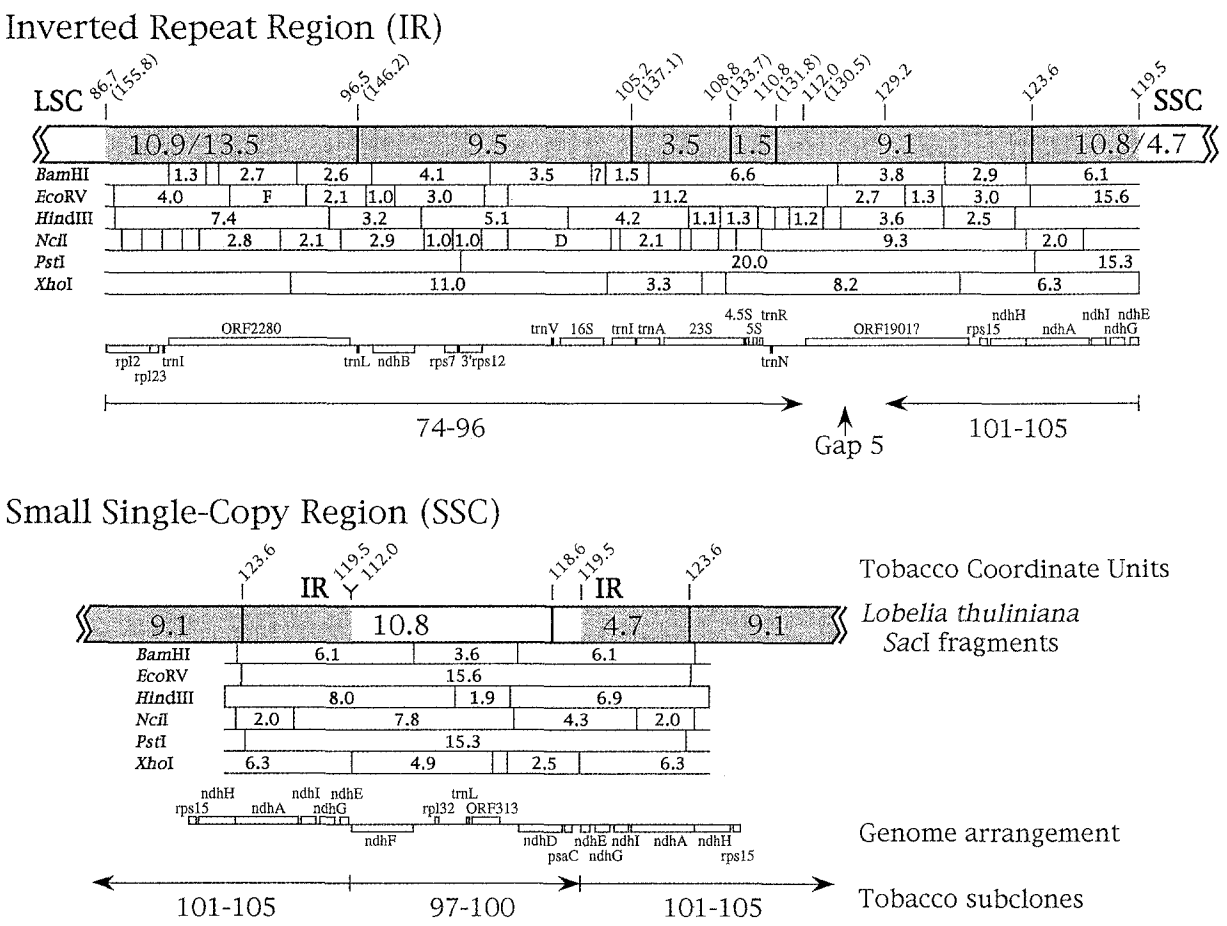

Fig. 3 (continued)

& al. 1993). These same rearrangements make cpDNA genome mapping using heterologous probes difficult because some probing results in Southern blot analysis yield ambiguous interpretation (see also KISHIMA & al. 1995, O'KANE 1995). The cloned cpDNA fragments of *Lobelia thuliniana* will facilitate future molecular studies of these families because (1) the SSC and adjacent portions of the IR regions are collinear in all *Lobeliaceae*, *Campanulaceae*, and *Cyphiaceae* studied to-date; (2) the limited sequence divergence gives stronger and more reliable probing results than, say, tobacco or lettuce probes; and (3) the fragments can be sub-cloned to create specialized probes that overlap or abut known rearrangement end-points in order to facilitate screening of rearrangement patterns in unstudied species in these families. The cpDNA clone bank from *Trachelium caeruleum* (*Campanulaceae*; COSNER & al. 1997) provides another useful set of probes, particularly for taxa closely related to *Trachelium* that share many of its rearrangements.

The IR is a nearly universal feature of land plant cpDNA (PALMER 1991, GOULDING & al. 1996), one copy having been lost only in conifers (LIDHOLM & al.

1988, STRAUSS & al. 1988), one group of papilionoid legumes (PALMER & THOMPSON 1981, PALMER & al. 1988b, WOLFE 1988, MILLIGAN & al. 1989, LAVIN & al. 1990), two related genera of *Geraniaceae* (*Erodium* and *Sarcocaulon*; CALIE & PALMER, unpubl. data, see PALMER 1991, DOWNIE & PALMER 1992a), and the parasite *Conopholis americana* (*Orobanchaceae*; DOWNIE & PALMER 1992a). The vast majority of land plants share the IR gene content and organization typified by tobacco (SHINOZAKI & al. 1986), which is usually little modified from the inferred ancestral condition of angiosperms (PALMER 1985, 1991; PALMER & al. 1987; DOWNIE & PALMER 1992a). A characteristic feature of the IR is the rRNA transcription unit (PALMER 1985, GOULDING & al. 1996), but the IR is capable of expansion and contraction on either side of this rRNA gene-cluster. At one extreme, among land plants, is the cultivated geranium (*Pelargonium* × *hortorum*) in which the IR is c. 76 kb due to expansions into both the SSC and LSC (with additional rearrangements due to inferred inversions; PALMER & al. 1987). At the other extreme, among land plants with the rRNA gene-cluster still contained in the IR, is the cultivated coriander (*Coriandrum sativum*) in which the IR is c. 12 kb (PALMER 1985). One conifer (*Pinus thunbergii*) is reported to retain a residual IR (0.5 kb) that contains *trn*I and the 3′ end of *psb*A, but does not contain the rRNA genes (TSUDZUKI & al. 1992, WAKASUGI & al. 1994). Given the highly rearranged genome of conifers, this may alternatively be explained as a recent duplication.

Contraction of the IR may simply result from deletion of DNA that spans one of the junctions with the LSC or SSC (GOULDING & al. 1996). Small contractions have been reported for several diverse species and may be part of the natural "ebb and flow" of the cpDNA IR (GOULDING & al. 1996). A larger contraction of the IR was reported for coriander (PALMER 1985), with *psb*A still present in the LSC, close to the J_{LA} boundary, but a segment of cpDNA including *rpl*2 (and presumably other genes not specifically probed) is now located on the LSC (rather than the IR) side of the J_{LB} boundary. If this contraction was due to deletion of one copy of the segment formerly present in the IR, then the deleted segment would have been removed from IR_{LA}. The entire IR_B was evidently deleted in conifers (STRAUSS & al. 1988, WAKASUGI & al. 1994), whereas IR_D (bounded by J_{LB} and J_{SA}) was evidently deleted in the lineage of papilionoid legumes (WOLFE 1988, HERDENBERGER & al. 1990), but in both cases the molecular events involved in the loss of one copy of the IR seem to be more complex than a simple deletion of one IR copy.

GOULDING & al. (1996) suggest that small IR expansions (< 100 bp) may occur via gene conversion, as illustrated by closely related species of *Nicotiana* and other dicot species. Grasses such as rice (HIRATSUKA & al. 1989) and maize (MAIER & al. 1995) contain both *trn*H and *rps*19 in the IR, and one might account for this by expansion of IR_{LA} (with attendant conversion of IR_{LB}) followed by expansion of IR_{LB} (with attendant conversion of IR_{LA}). Rice and maize also show small differences in the positions of J_{SA} and J_{SB}. GOULDING & al. (1996) also propose a mechanism involving double-strand DNA breaks to account for the large expansions of the IR observed in some groups of plants. A large expansion of IR_{LA} has been reported for four genera of ferns (STEIN & al. 1992), which also have two inversions in the IR. Large expansions of IR_{LB} have been reported for

Nicotiana acuminata (SHEN & al. 1982, see also GOULDING & al. 1996), geranium (PALMER & al. 1987), five related genera of *Ranunculaceae* (HOOT & PALMER 1994; see also JOHANSSON & JANSEN 1993), and two related genera of *Berberidaceae* (KIM & JANSEN 1994). Expansions of IR_{SA} have been reported for the *Gramineae* (HIRATSUKA & al. 1989, MAIER & al. 1995), three species of buckwheat (KISHIMA & al. 1995), and *Trachelium* (*Campanulaceae*; COSNER & al. 1997). A large expansion of the IR into the SSC has also been reported for geranium (PALMER & al. 1987), but it was not determined which of the two copies was involved.

Expansion of IR_{SA} was one of three cpDNA rearrangements shared by 18 genera of *Campanulaceae* (*Adenophora*, *Asyneuma*, *Campanula*, *Codonopsis*, *Cyananthus*, *Edraianthus*, *Jasione*, *Legousia*, *Merciera*, *Musschia*, *Petromarula*, *Platycodon*, *Prismatocarpus*, *Roella*, *Symphyandra*, *Trachelium*, *Triodanus*, *Wahlenbergia*; COSNER 1993); the other two rearrangements being the apparent deletion of *acc*D and an inversion affecting *rbc*L, *atp*B, and *atp*E (54.9–59.3 TCU; tobacco subclones 41–44). COSNER & al. (1997) present three alternative models (labeled A, B, and C) to account for the extensive cpDNA genome rearrangements observed in *Trachelium caeruleum*. Comparisons of the *Campanulaceae* and *Lobeliaceae* cpDNA genomes suggest that the expansion of IR_{SA} and loss of *acc*D are shared events that predate divergence of these two families, and this strengthens the argument of COSNER & al. (1997) against model B as a plausible alternative.

The *Lobeliaceae* are sometimes treated as subfam. *Lobelioideae* in the *Campanulaceae* s. l. (along with the *Campanuloideae* and *Cyphioideae*; see WIMMER 1943, 1953, 1968; although LAMMERS (1998) notes that the name *Cyphocarpoideae* has priority over *Cyphioideae*). The *Lobeliaceae* and *Campanulaceae* s. str. are probably both monophyletic, but the heterogeneous cyphioid genera (*Cyphia*, *Cyphocarpus*, *Nemacladus*, *Parishella*, and *Pseudonemacladus*) remain problematic and have generally been retained in the family *Cyphiaceae*, which is unlikely to be monophyletic (LAMMERS 1992). Phylogenetic analyses using morphology and cpDNA *rbc*L sequence data show conflicting relationships among representatives of these three families (COSNER & al. 1994, GUSTAFSSON 1996). The distinctive cpDNA rearrangements in these families constitute useful molecular markers that should eventually help delimit the *Lobeliaceae* and *Campanulaceae* and resolve the placement of the cyphioid genera. The absence of the IR/SSC rearrangement in *Sphenoclea* provides additional support for the exclusion of this genus from the *Campanulaceae* s. l. (LAMMERS 1992, COSNER & al. 1994, GUSTAFSSON & al. 1996). It will be of interest in the future to survey the presence or absence of *acc*D and the IR/SSC rearrangement in other families such as the *Pentaphragmataceae*, *Stylidiaceae*, and *Donatiaceae* that may be closely related to the *Campanulaceae*, *Cyphiaceae*, and *Lobeliaceae*.

Mapping of the *Lobelia thuliniana* cpDNA genome confirms our earlier results using heterologous probes regarding the genome arrangement in the giant lobelias. Our earlier map estimates indicated the presence of c. 1.2 kb of non-hybridizing DNA in the region formerly occupied by *acc*D, suggesting a deletion of 1.6 kb (KNOX & al. 1993), but our new map estimates suggest that as much as 1.7 kb of non-hybridizing DNA may be present in Gaps 1 and 2, and that the putative deletion may be as small as 1.1 kb. It is not possible to determine the extent to

which non-coding DNA retains sufficient sequence homology with heterologous probes to yield positive probing results, so a more precise characterization of this putative deletion must await nucleotide sequencing of these gaps (see also SASAKI & al. 1989, SMITH & al. 1991, DOYLE & al. 1995). Previous results (KNOX & al. 1993) suggest that all *Lobeliaceae* lack *acc*D. The loss of *acc*D in cpDNA of the *Gramineae* and related *Anarthriaceae*, *Centrolepidaceae*, *Cyperaceae*, *Eriocaulaceae*, *Flagellariaceae*, *Joinvilleaceae*, *Juncaceae*, and *Restionaceae* (HIRATSUKA & al. 1989, DOWNIE & PALMER 1992a, MAIER & al. 1995, KATAYAMA & OGIHARA 1996) is thought to result from a transfer of function (i.e. gene substitution) from the prokaryotic form of the gene to a nuclear-encoded eukaryotic form of the gene, which is responsible for their susceptibility to graminicides (KONISHI & SASAKI 1994). The non-photosynthetic parasite *Epifagus virginiana* has an extremely reduced chloroplast genome but retains *acc*D as one of, at most, only four genes not involved in the gene-expression apparatus (WOLFE & al. 1992). The cpDNA *acc*D gene may also have been lost independently in species of *Annonaceae*, *Geraniaceae*, *Leguminosae*, and *Oleaceae* (DOWNIE & PALMER 1992a, DOYLE & al. 1995; but see discussion therein).

Lobelia thuliniana seems to have sufficient non-coding cpDNA sequence homology with other members of the *Lobeliaceae* that small fragments in putatively non-coding regions are resolved in probing experiments (E. B. KNOX, unpubl. data). Although the cpDNA genome is generally tightly packed with genes that yield strong probing results in Southern blot analyses across a wide taxonomic spectrum, our results underscore the need to use probes from closely related species and to carefully map regions (taking the location of genes into consideration) for which negative probing results suggest deletions. For example, the slow rate of nucleotide evolution in the IR (WOLFE & al. 1987; PALMER 1991; DOWNIE & PALMER 1992b, 1994; MAIER & al. 1995) means that strong probing results are usually obtained even in the non-coding region between 3′*rps*12 and *trn*V (100.9–102.5 TCU; tobacco subclone 90), but small fragments in the non-coding region between *rpl*32 and *trn*L (115.1–116.1 TCU; tobacco subclone 98) in the SSC and the non-coding segments of the region between *rpo*B and *psb*D (27.5–34.5 TCU; tobacco subclones 25–29) in the LSC are frequently not resolved by heterologous probes with significant sequence divergence (see also PALMER & STEIN 1986, KISHIMA & al. 1995).

Although sequence data from cpDNA genes have become important lines of evidence for phylogenetic reconstruction in plants (e.g. CHASE & al. 1993), Southern blot analysis remains a cost-effective approach for screening both restriction-site variation and genome arrangement. When present, cpDNA genome rearrangements provide important phylogenetic markers that are rarely homoplasious (DOWNIE & PALMER 1992a; but see DOWNIE & PALMER 1994 and HOOT & PALMER 1994). Taken together, these lines of cpDNA molecular evidence should yield congruent phylogenetic estimates which, in broader comparisons with nuclear DNA and morphologically-based phylogenetic analyses, should continue to enrich our understanding of plant evolution.

We thank the Tanzanian Commission for Science and Technology, the Ugandan National Research Council, the National Museums of Kenya, the Institute Zairois pour la

Conservation de la Nature, the Office Rwandais du Tourisme et des Parcs Nationaux, and Addis Abeba University and the Ethiopian Ministry of Foreign Affairs for permission to conduct research in their respective countries. T. AYERS, P. BERRY, C. DEPAMPHILIS, J. GAME, T. GIVNISH, A. HAMILTON, A. KLOSTOBOR, T. LAMMERS, J. SMITH, K. SYTSMA, the Missouri Botanical Garden, the Pacific Tropical Botanical Garden, the Royal Botanic Gardens, Kew, and the University of California Botanical Garden kindly provided plant material used in this work. We thank R. JANSEN for useful comments on the manuscript. This work was supported in part by National Science Foundation grants BSR-8800487, BSR-8901123, and BSR-8996262.

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Addresses of the authors: ERIC B. KNOX, Department of Biological Sciences, Rutgers University, 101 Warren Street, Newark, New Jersey 07102, USA. – JEFFREY D. PALMER, Department of Biology, Jordan Hall, Indiana University, Bloomington, Indiana 47405, USA.

Accepted August 26, 1997 by B. L. TURNER