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Expansion and Contraction of the Chloroplast Inverted Repeat in Apiaceae Subfamily Apioideae

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ABSTRACT. Chloroplast DNA (cpDNA) restriction site maps for 113 species of Apiaceae (Umbelliferae) and the allied families Araliaceae and Pittosporaceae were constructed for two enzymes and examined for variation in position of J_{LB}, the junction between the large single copy and inverted repeat regions that is typically contained within the ribosomal protein *S10* operon. With the exception of one large clade in Apiaceae subfamily Apioideae, all species possess a J_{LB} indistinguishable from that found in the vast majority of angiosperms. Within this large clade, however, at least one expansion and seven different contractions of the IR relative to the tobacco J_{LB} were detected, each ranging in size from ~1–16 kb. Five of the junction shifts are parsimony informative, and three support major clades delimited in earlier phylogenetic studies. In light of cladograms based on previous studies of restriction site and DNA sequencing data, the IR appears to have expanded and contracted a minimum of ten times during the evolution of Apioideae, with several presumably identical size variants occurring in parallel. The frequency and large size of J_{LB} shifts in Apioideae cpDNAs are unprecedented among angiosperms, indicating that the subfamily represents a model system to study the mechanisms leading to large-scale expansions and contractions of the IR.

The chloroplast genomes of the majority of photosynthetic land plants are highly conserved in size, structure, gene arrangement, and content (Palmer 1985a, 1985b, 1991; Palmer and Stein 1986; Downie and Palmer 1992b). Their hallmark is the presence of two large duplicate regions in reverse orientation known as the inverted repeat (IR), which separate the remainder of the circular molecule into a large single-copy (LSC) region of about 87 kilobase pairs (kb) and a small single-copy (SSC) region of about 18 kb. Excluding some papilionoid legumes (Palmer et al. 1988; Lavin et al. 1990), all conifers (Lidholm et al. 1988; Strauss et al. 1988; Raubeson and Jansen 1992), and some species of Geraniaceae and Orobanchaceae (Downie and Palmer 1992b), which lack one copy of the IR, most angiosperms possess an IR that ranges between 22 and 26 kb in size (Palmer 1985b; Downie and Palmer 1992b). The 495-bp residual IR reported for black pine lacks the rRNA gene cluster (Tsudzuki et al. 1992) and has alternatively been explained as a repetitive sequence resulting from a recent duplication (Knox and Palmer 1999). Given its near universal presence among land plants, the IR has been interpreted as an ancestral feature that was lost several times independently (Palmer 1991).

Of the two equimolar structural isomers existing for chloroplast DNA (cpDNA; Palmer 1983), the structure most commonly illustrated follows the convention used for tobacco in which one copy of the IR (flanked by single-copy genes *psbA* and ORF1901) is designated as IR_A and the other copy (flanked by single-copy genes *rps19* and *ndhF*) is designated as IR_B (Fig. 1; Shinozaki et al. 1986). The junctions between the LSC region and each of these IR copies are designated as J_{LA} (LSC/IR_A) and J_{LB} (LSC/IR_B) (Fig. 1), and the junctions flanking the SSC region are designated as J_{SA} and J_{SB} (Shinozaki et al. 1986). In most angiosperms, J_{LB} lies within the ribosomal protein *S10* operon in a more or less fixed position within or near the *rps19* gene (Palmer 1985b; Goulding et al. 1996). Both IR copies are identical in nucleotide sequence and encode, with few exceptions, the rRNA transcription unit and the homolog of tobacco ORF2280, the largest chloroplast gene of most land plants (Downie et al. 1994).

Most angiosperm chloroplast genomes range between 135 and 160 kb in size (Palmer 1985b). Variation in the size of the molecule is due most typically to the expansion or contraction of the IR into or out of adjacent single-copy regions (i.e., the movement of J_{LB} and other IR-single copy junctions), and/or changes in sequence complexity due to insertions or deletions of unique sequences. At one extreme is the c. 217 kb-genome of geranium (*Pelargonium* × *hortorum* Bailey) possessing a greatly enlarged IR of 76 kb, almost three times the size found in most angiosperms (Palmer et al. 1987a). Here the IR has expanded into both LSC and SSC regions, and thus many protein-coding genes pre-

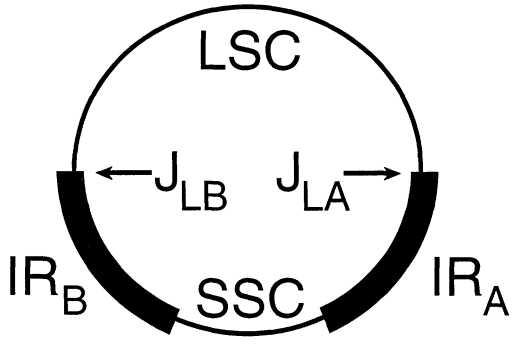

FIG. 1. Structural organization of a typical land plant chloroplast genome. The inverted repeat regions IR_A and IR_B (thick lines) divide the rest of the circular genome into large single-copy (LSC) and small single-copy (SSC) regions. Junctions between the IRs and single-copy regions, designated J_{LA}, J_{LB}, J_{SA}, and J_{SB} according to Shinozaki et al. (1986), are illustrated. This illustration portrays the chloroplast genome in only one of its two orientations (in the other, the single-copy regions are reversed in polarity; Palmer 1983).

sent only once in most other plants are duplicated in geranium. At the other extreme (provided that the IR has not been deleted in its entirety) is coriander (*Coriandrum sativum*), whose IR has been reported to be less than half the normal size (Palmer 1985b) due, presumably, to deletion of a portion of the IR adjacent to J_{LA} (Knox and Palmer 1999). Within this range, a number of other IR size variants have been reported relative to tobacco, including a 12-kb expansion in *Nicotiana acuminata* Hook. (Shen et al. 1982; Goulding et al. 1996), an 11.5-kb expansion in two related genera of Berberidaceae (Kim and Jansen 1994), an 11-kb expansion in allied Lobeliaceae, Campanulaceae, and Cyphiaceae (Knox and Palmer 1999), a 4-kb expansion in six related genera of Ranunculaceae (Johansson and Jansen 1993; Hoot and Palmer 1994), a 4–5-kb expansion in three related species of *Fagopyrum* (Kishima et al. 1995; Aii et al. 1997), and a probable 6.5 kb contraction in *Cuscuta* (Bömmer et al. 1993; Downie et al. 1994). Smaller contractions and extensions of the IR (<100 bp) occur frequently among angiosperms, with differences apparent even among closely related species (Goulding et al. 1996). These small endpoint differences, which can affect all border positions, have been confirmed by DNA sequencing for maize and rice (Hiratsuka et al. 1989; Maier et al. 1990, 1995), *Nicotiana* (Zurawski et al. 1984; Goulding et al. 1996), and several other dicot genera (Goulding et al. 1996).

The IRs of land plants can therefore fluctuate in size, duplicating genes or other DNA segments that would otherwise be single-copy or losing duplicated sequences to single-copy regions. Either IR copy can be lost and the positions of all four junctions can vary (Goulding et al. 1996; Cosner et al. 1997; Knox and Palmer 1999). Moreover, given the presence of a second structural isomer of cpDNA (Palmer 1983) where the SSC and LSC regions are in different relative orientations and the structural rearrangements that can accompany expansion or contraction events (e.g., Cosner et al. 1997; Knox and Palmer 1999), the IR should not be viewed as a region that simply expands and contracts but rather as a region prone to much and sometimes quite complex variation.

This variability in IR junction position can be exploited for phylogenetic purposes, as specific large expansions of the IR have already served to demarcate monophyletic groups (discussed above) and small expansions or contractions tend to have similar endpoints in closely related species (Goulding et al. 1996). In a recent cpDNA restriction site mapping study of Apiaceae (Umbelliferae), we reported four different IR size classes, attributable to variation in position of J_{LB} (Plunkett and Downie 1999). In the most extreme arrangement, the position of J_{LB} differed by ~17 kb. This variation, representing one expansion and three contractions relative to the tobacco J_{LB}, was restricted to Apiaceae subfamily Apioideae, a taxon whose suprageneric classification has been elusive (Plunkett et al., 1996b; Downie et al., 1998, 2000; Plunkett and Downie 1999). Thus, specific plastid structural rearrangement data might serve to illuminate apioid phylogeny or to provide additional support for otherwise weakly-supported clades. The large size of the probes employed in that study, however, prevented us from mapping this junction more precisely. Given the rarity of numerous IR-junction changes of this magnitude (particularly within a single family) and the potential utility of structural rearrangements as phylogenetic characters (Downie and Palmer 1992b), we herein examine an expanded sample of taxa from Apiaceae using smaller probes to characterize more accurately the nature and distribution of these structural rearrangements. To assess the extent of J_{LB} mobility during the evolution of the group, we also consider their distribution in light of phylogenetic hypotheses inferred by earlier restriction site and DNA sequencing studies.

Materials and Methods

A total of 113 species was examined for expansion or contraction of the cpDNA IR (Table 1), representing 95 species (in 75 genera) from Apiaceae subfamily Apioideae, seven species (six genera) from Apiaceae subfamily Hydrocotyloideae, and six species (five genera) from Apiaceae subfamily Saniculoideae. Five outgroup genera (three species from Araliaceae and two from Pittosporaceae) were also examined. Phylogenetic analysis of molecular data (Downie and Palmer 1992a; Olmstead et al. 1992, 1993; Chase et al. 1993; Plunkett et al. 1996a) support traditional taxonomic evidence (Dahlgren 1980; Cronquist 1981) in suggesting that these two families are closely related to Apiaceae.

Total genomic DNA was extracted from fresh or dried leaf material using the modified CTAB method of Doyle and Doyle (1987), followed by ultracentrifugation in cesium chloride/ethidium bromide gradients (Sambrook et al. 1989). The purified DNAs were digested singly with each of two restriction endonucleases, *Bam*HI and *Hin*dIII. The resulting DNA fragments, along with size markers (two lanes of lambda phage DNA double-digested with *Eco*RI and *Hin*dIII and a single lane of tobacco cpDNA digested with either *Bam*HI or *Hin*dIII), were separated electrophoretically in 1.0% agarose gels. These fragments were then transferred bidirectionally onto MagnaCharge (Micron Separations, Inc., Westborough, Massachusetts) nylon filters (Southern 1975) and probed with 19 subclones derived from tobacco cpDNA. Following hybridization, the filters were washed in 2X SSC, 0.5% SDS twice for 5 min at room temperature and twice for 60 min at 65°C. After visualization by autoradiography, fragment sizes were estimated by comparison to the size markers.

We have focused on examining J_{LB} because our previous survey of restriction site variation in Apiaceae detected variability in its position (Plunkett and Downie 1999). Each of 19 cloned restriction fragments of tobacco cpDNA (provided by J. Palmer, Indiana University, Bloomington) were labeled with ^{32}P by random priming and used as probes in filter hybridizations. These probes ranged in size from 209 to 3,269 bp (averaging 923 bp) and were much smaller than the seven "SolClones" (1,112–5,164 bp, averaging 2,468 bp; Sugiura et al. 1986; Olmstead and Palmer 1992) used as probes in our initial investigation of the same region. The 19 probes are labeled from 69 to 90 (Fig. 2), following the nomenclature used by Palmer et al. (1994), and together represent the regions flanking the J_{LB} [corresponding to tobacco coordinates 85,250–104,801 of Shinozaki et al. (1986) and comprising some 19.5 kb of sequence]. Probes 70–71 and 76–77 were each used in combined hybridizations; probe 76 was also used singly. Probe 89, specific for the 3′-*rps*12 gene, was not used as it failed to hybridize to all examined taxa including tobacco. Of the 19 probes employed, three contain all or parts of more than two genes, three others contain all or part of two genes, and 13 are entirely internal to or contain only part of a single gene. Six of these last 13 hybridize to either 5′ or 3′ gene portions, and can thus be used to confirm differences in gene order or the direction of transcription through differential hybridization patterns. Within the region surveyed, genes *rpl*2, *ndh*B, and 3′-*rps*12 each contain introns. Probe 75 is internal to the *rpl*2 intron, whereas probe 86 is largely specific for the *ndh*B intron. Six probes (78–83) are specific for the tobacco ORF2280 region. The small sizes of the probes permit the detection of small rearrangement events that are often undetected when larger fragments are used (such as small shifts in IR endpoints, gene and intron losses, and inversions), and reduce the uncertainty inherent in maps inferred solely by single enzyme digests of cpDNA (Downie and Palmer 1992b).

Fine-scale restriction site maps for each of the two enzymes for all 113 species were constructed for the entire 19.5 kb region flanking J_{LB} using these 19 small probes. Mapping was facilitated by comparisons with maps constructed during our earlier study of the entire chloroplast genome using 14 enzymes including *Bam*HI and *Hin*dIII (Plunkett and Downie 1999). For 14 species included in both studies, the sizes of the IR, SSC, and LSC regions, as well as their entire chloroplast genomes, were estimated. The location of the junction between the IR and the LSC regions was inferred by the presence of a characteristic "overlapping" fragment pattern. Because restriction sites found within the IR occur symmetrically in both copies (Palmer 1985a), probes that hybridize to restriction fragments located entirely within the IRs will produce identical banding patterns. The equal hybridization of a single probe to two co-migrating fragments implies the presence of an IR. Restriction fragments that overlap the two LSC-IR margins are characterized by one common restriction site (occurring symmetrically in each of the IR segments) and by one different site (occurring asymmetrically within the ends of the adjacent single-copy region). Therefore, a single probe will often hybridize to two distinct

bands resulting from fragments spanning both J_{LA} and J_{LB}. Differences in strength of hybridization between fragments from a single probe, as well as the differential hybridization of these fragments and their presence among flanking probes, help define the endpoint of the IR. This method, however, provides only an estimate of junction placement; its actual position is contained somewhere within the region circumscribed by a particular overlapping probe. Larger probes provide greater ambiguity in junction placement (e.g., probe 90), whereas the smaller probes (e.g., probe 73) provide greater accuracy.

Results

The small sizes of the probes used herein permit a fine level of inference regarding IR structure, organization, and content. Each of the 19 tobacco probes hybridized strongly and in a colinear manner to all 113 accessions. ORF2280, the largest gene in the plastid genomes of most land plants, is particularly prone to length mutation and exists as a pseudogene in several independent lineages (Downie et al. 1994, 1997). In this study, no major length variants within the ORF or anywhere else within the IR were detected. Both introns in genes *rpl*2 and *ndh*B were present, as ascertained by probes specific or nearly specific for these regions; the restriction site maps indicate that the 3′-*rps*12 intron is likely present as well because there was no evidence of deletion within this region. Therefore, within the limits of detection of our mapping studies, the chloroplast genomes of Apiaceae, Araliaceae, and Pittosporaceae are identical in genome organization and content, at least in the vicinity of the LSC-IR junction in IR_B, to that of tobacco; the only major difference in structure was the position of J_{LB} in some taxa.

With the exceptions of the apioid species Astomaea sessilifolium and Conioselinum chinense, unambiguous restriction site maps of the J_{LB} region could be constructed for all taxa. These maps reveal that all representatives sampled from Pittosporaceae (two species), Araliaceae (three species), and Apiaceae subfamilies Hydrocotyloideae (seven species) and Saniculoideae (six species), as well as 39 of the 95 species examined from Apiaceae subfamily Apioideae, possess a J_{LB} indistinguishable from that found in tobacco and the vast majority of other flowering plants (denoted hereafter as a "type A" or "typical" junction; see Table 2; Fig. 2). Among the remaining apioid taxa (but excluding *Astomaea* and *Conioselinum*), eight other junction types are apparent; these represent one expansion (type B) and seven distinct contractions (types C—I) in IR size relative to tobacco. For those species exhibiting the type B junction, the IR has expanded by ~1.1 kb. Consequently, protein coding genes *rps3*, *rpl22*, and *rps19* that are present only once in most other plants are duplicated in these species. The seven IR contractions (types C—I) range in size from ~ 0.9–16.1 kb and represent the removal of duplicated sequences from the genome. The genes occupying these deleted segments, formerly located within the IR, are now located on the LSC side of the J_{LB} boundary. As such, the deleted segments have been removed from I_{RA}. The difference in position between junction types B and I represents ~17 kb. In *Coriandrum*, where the most extreme IR contraction is represented, the gene *rpl2* (normally located near the terminus of the IR) is a single-copy gene some 16 kb away from the end of the repeat. The LSC-IR junctions in *Astomaea* and *Conioselinum* could not be determined with the same degree of certainty as in the other species because their restriction maps suggested either junction type A or B. Table 2 summarizes these nine junction types, indicating the direction and size of the junction shifts and the coding regions potentially affected by them.

For 14 species that were included in our earlier study of cpDNA restriction site variation (Plunkett and Downie 1999), the sizes of the entire chloroplast genome as well as each of their constituent structural regions (i.e., LSC, SSC, and IR) are provided (Table 3). These species represent each of the major taxonomic groups outlined in our previous investigations as well as seven of the nine inferred junction types (J_{LB} types C and G were not represented as the species possessing these types were not included in our earlier study). Genome size estimates were derived by averaging the values based on separate *Bam*HI and *Hin*dIII restriction site maps. Using the methods described above, very large fragments (> 15 kb) are difficult to size accurately and very small fragments (< 0.25 kb) may not be detected; thus, the sizes provided herein must be considered approximate. The total size of the chloroplast genome ranged from 152.7–157.0 kb (averaging 154.5 kb) among taxa with junction type A (i.e., the typical J_{LB}). Within this same group, the average size of one copy of the IR was 25.6 kb, that of the LSC was 83.9 kb, and that of the SSC 19.4 kb; these values are within 0.2–2.8 kb of the sizes reported for tobacco cpDNA (Shinozaki et al. 1986). The 79.9 kb LSC region of *Carum carvi* (J_{LB} type B)

TABLE 1. Accessions of Apiaceae and allied families examined for cpDNA IR expansion/contraction. Herbarium acronyms follow Holmgren et al. 1990. "UIUC" = University of Illinois at Urbana-Champaign; BG = botanic (al) garden; "cult." = cultivated; "#" = accession number.

Taxon	Source
Pittosporaceae	
Hymenosporum flavum F. J. Muell.	cult. UIUC, from seeds obtained from North Coast Regional BG, Coffs Harbour, Australia, *Downie 836* (ILL), *Plunkett 1463* (ILL)
Pittosporum revolutum Aiton	cult. UIUC, from seeds obtained from North Coast Regional BG, Coffs Harbour, Australia, *Downie 829* (ILL), *Plunkett 1462* (ILL)
Araliaceae	
Aralia spinosa L.	cult. Missouri BG (#895974)
Fatsia japonica (Thunb.) Decne. & Planch.	cult. Royal BG Edinburgh, Scotland (#19687549)
Trevesia sundaica Miq.	cult. Missouri BG (# 801619)
Apiaceae: Hydrocotyloideae	
Azorella trifurcata (Gaertn.) Pers.	cult. Royal BG Edinburgh, Scotland (#19760821)
Bolax gummifera (Lam.) Spreng.	cult. Royal BG Edinburgh, Scotland (#19361025)
Centella asiatica (L.) Urb.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-1649)
Centella erecta (L. f.) Fern.	USA, Florida, Wakulla Co., *Godfrey s.n.* (UC); cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-1477)
Didiscus pusilla DC.	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 35* (ILL)
Eremocharis fruticosa Phil.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2382), originally from Chile, Antofagasta, Quebrada Coquimbo, Taltal, *Dillon & Teillier 5082* (UC)
Klotzschia rhizophylla Urb.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2414), originally from Brazil, Minas Gerais, Serra do Cipo, *Pirani 12909* (UC);
Apiaceae: Saniculoideae	
Astrantia major L.	cult. Royal BG Edinburgh, Scotland (#19861407), originally from Switzerland, *Schilling 2937* (E);
Eryngium cervantesii Delar. f.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2443)
Eryngium varifolium Coss.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. s.n.)
Hacquetia epipactis (Scop.) DC.	cult. Royal BG Edinburgh, Scotland (#19694625)
Petagnaea saniculifolia Guss.	cult. Royal BG Edinburgh, Scotland (#19695641)
Sanicula canadensis L.	USA, Illinois, Champaign Co., Urbana, *Downie 737* (ILL)
Apiaceae: Apioideae	
Aciphylla aurea W. R. B. Oliv.	cult. Royal BG Edinburgh, Scotland (#19712219) originally from New Zealand
Aegokeras caespitosa (Sibth. & Sm.) Raf.	cult. Royal BG Edinburgh, Scotland (#19100154), originally from Univ. of Cambridge BG, England
Aethusa cynapium L.	cult. UIUC, from seeds obtained from Univ. of Oldenberg BG, Germany, *Downie 146* (ILL)
Ammi majus L.	cult. UIUC, from seeds obtained from Jardin botaniques de Nancy, France, *Downie 252* (ILL)
Anethum graveolens L.	cult. UIUC, from seeds obtained from Univ. of Oldenberg BG, Germany, *Downie 157* (ILL)
Angelica archangelica L.	cult. UIUC, from seeds obtained from Univ. of Joensuu BG, Finland, *Downie 78* (ILL)
Angelica dahurica (Hoffm.) Franch. & Sav.	cult. Univ. of California BG, Berkeley (#88.0678), originally from China
Angelica decursiva (Miq.) Franch. & Sav.	cult. UIUC, from seeds obtained from Shanghai BG, China, *Downie 359* (ILL)

TABLE 1. Continued.

Taxon	Source
Angelica polymorpha Maxim.	cult. Univ. of California BG, Berkeley (#90.0662), originally from Japan, Miyazaki, Kyushu, *McNamara et al. 264* (UC)
Anginon rugosum (Thunb.) Raf.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2399) originally from South Africa, West Cape, *Batten 1018* (UC)
Anisotome aromatica Hook. f.	cult. Royal BG Edinburgh, Scotland (#19881687), originally from New Zealand, South Island, Canterbury, *Corden 29* (E);
Anthriscus cerefolium (L.) Hoffm.	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Downie 24* (ILL)
Apium graveolens L.	cult. UIUC, from seeds obtained from Jardin botaniques de Nancy, France, *Downie 262* (ILL)
Arracacia aegopodioides (Humb.) J. M. Coult. & Rose	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2408)
Arracacia bracteata J. M. Coult. & Rose	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2412)
Arracacia brandegei J. M. Coult. & Rose	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2045), originally from Mexico, Baja California del Sur, *Breedlove 43405* (UC)
Arracacia pringlei J. M. Coult. & Rose	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2330)
Arracacia tolucensis (Humb.) Hemsl.	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2124)
Astomaea sessilifolium (DC.) Rauschert	Jordan, Jarash, Ain El-Deek, *Lahham & El-Oqlah 21* (Yarmouk Univ. Herbarium)
Astrodaucus orientalis (L.) Drude	cult. UIUC, from seeds obtained from Research Institute of Forests and Rangelands, Iran, *Lee 43* (ILL)
Berula erecta (Huds.) Coville	cult. UIUC, from seeds obtained from Univ. of Oldenberg BG, Germany, *Downie 150* (ILL)
Berula thunbergii (DC.) H. Wolff	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2453), originally from Ethiopia
Bifora radians M. Bieb.	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 28* (ILL)
Bunium elegans (Fenzl) Freyn	Jordan, Ajlun, near the Community College, *Lahham & El-Oqlah 9* (Yarmouk Univ. Herbarium)
Bupleurum chinense DC.	cult. UIUC, from seeds obtained from Shanghai BG, China, *Downie 409* (ILL)
Bupleurum falcatum L.	cult. Moscow State Univ. BG, Russia, from seeds obtained from Wroclaw BG, Poland
Bupleurum ranunculoides L.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 94* (ILL)
Bupleurum rotundifolium L.	cult. UIUC, from seeds obtained from Jardin botanique de Caen, France, *Downie 304* (ILL)
Capnophyllum dichotomum (Desf.) Lag.	cult. UIUC, from seeds obtained from Jardin botanique National de Belgique, Belgium, *Downie 285* (ILL)
Carlesia sinensis Dunn	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2401), originally from China, Hort. Nanjing
Carum alpinum Benth. & Hook. f.	cult. UIUC, from seeds obtained from Univ. of Turku, Finland, *Downie 424* (ILL)
Carum carvi L.	cult. UIUC, from seeds obtained from Jardin botaniques de Nancy, France, *Downie 243* (ILL)
Caucalis platycarpos L.	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 75* (ILL)
Chaetosciadium trichospermum (L.) Boiss.	Jordan, Um-Qais near Irbid, *Lahham & El-Oqlah 4* (Yarmouk Univ. Herbarium)
Chymsydia colchica (Albov) Woronow ex Grossh.	Georgia, Mt. Kvira, *Pimenov 1489* (MW); cult. Moscow State Univ. BG, Russia
Cicuta virosa L.	cult. UIUC, from seeds obtained from Univ. of Joensuu BG, Finland, *Downie 75* (ILL)
Cnidium officinale Makino	cult. UIUC, from seeds obtained from Institut für Plflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Downie 830* (ILL)

TABLE 1. Continued.

Taxon	Source
Cnidium silaifolium (Jacq.) Simonk.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Plunkett 1470* (ILL)
Coaxana purpurea J. M. Coult. & Rose	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2411), originally from Mexico, Oaxaca, *Breedlove 72745* (UC)
Conioselinum chinense (L.) Britton, Stern, & Poggenb.	cult. Univ. of California BG, Berkeley (#83.0114), originally from USA, California, San Mateo Co., San Bruno Mtn., *Raiche 30046* (UC)
Conium maculatum L.	cult. UIUC, from seeds obtained from Jardin botaniques de Nancy, France, *Downie 241* (ILL)
Conium maculatum L.	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Downie 16* (ILL)
Coriandrum sativum L.	cult. UIUC, from seeds obtained from Johannes Gutenburg Univ., Germany, *Downie 65* (ILL)
Coulterophytum laxum Robins	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-1650), originally from Mexico, Michoacán, *Iltis 298 & Cochrane* (UC)
Crithmum maritimum L.	cult. UIUC, from seeds obtained from Quail BGs, California, *Downie 345* (ILL)
Cryptotaenia canadensis (L.) DC.	USA, Illinois, Champaign Co., Urbana, *Downie 817* (ILL)
Cryptotaenia japonica Hassk.	cult. Univ. of California BG, Berkeley (#90.0891), originally from Japan, Honshu Island, Koyosan area, *McNamara et al. 90* (UC)
Cuminum cyminum L.	cult. UIUC, from seeds obtained from commercial source, *Lee 120* (ILL)
Cymopterus globosus (S. Watson) S. Watson	USA, Nevada, Washoe Co., *Lyons-weiler s.n.* (RENO)
Daucus carota L.	USA, Illinois, Champaign Co., Urbana, *Downie 741* (ILL)
Daucus montanus Humb. & Bonpl.	cult. Univ. of California BG, Berkeley (#94.0563), originally from Argentina
Enantiophylla heydeana J. M. Coult. & Rose	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2251), originally from Mexico, Jalisco, *Iltis et al. 3187* (UC)
Endressia castellana Coincy	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2184)
Falcaria vulgaris Bernh.	Jordan, Irbid, Yarmouk Univ. Campus, *Lahham & El-Oqlah 2* (Yarmouk Univ. Herbarium)
Ferula assa-foetida L.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 490* (ILL)
Ferula communis L.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 112* (ILL)
Foeniculum vulgare P. Mill.	cult. UIUC, from seeds obtained from National BGs, Glasnevin, Ireland, *Downie 187* (ILL)
Heracleum lanatum Michx.	USA, California, Marin Co., Muir Woods, *Downie 579* (ILL)
Heteromorpha arborescens (Spreng.) Cham. & Schltdl.	cult. UIUC, from seeds obtained from Real Jardín Botánico, Madrid, Spain, *Downie 42* (ILL)
Laserpitium hispidum M. Bieb.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 120* (ILL)
Lecokia cretica (Lam.) DC.	Jordan, Ajlun, near Schtafeenah, *Lahham & El-Oqlah 7* (Yarmouk Univ. Herbarium)
Levisticum officinale W. D. J. Koch	cult. UIUC, from seeds obtained from Univ. of Oldenberg BG, *Downie 161* (ILL)
Ligusticum scoticum L.	USA, Massachusetts, Plymouth Co., *Raiche 40411* (UC); cult. Univ. of California BG, Berkeley (#84.0620)
Lomatium californicum (Nutt.) Mathias & Constance	USA, California, Napa Co., *Plunkett 1310* (WS)
Mathiasella bupleuroides Constance & C. Hitchcock	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2447), originally from Mexico, Nuevo Leon, Cerro El Viejo, *Hinton et al. 22234* (UC)
Meum athamanticum Jacq.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 482* (ILL)
Myrrhis odorata (L.) Scop.	cult. Univ. of California BG, Berkeley (#89.1236), originally from Europe
Notopterygium incisum Ting ex Ho-T Chang	cult. UIUC, from seeds obtained from Shanghai BG, China, *Downie 400* (ILL)

TABLE 1. Continued.

Taxon	Source
Oenanthe banatica Heuff.	cult. UIUC, from seeds obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 476* (ILL)
Oenanthe fistulosa L.	cult. UIUC, from seeds obtained from Univ. of Oldenberg BG, Germany, *Downie 165* (ILL)
Orlaya kochii Heywood	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Downie 20* (ILL)
Osmorhiza chilensis Hook. & Arn.	cult. Univ. of California BG, Berkeley, originally from USA, California, Alameda Co.
Pastinaca sativa L.	cult. UIUC, from seeds obtained from Jardin botaniques de Nancy, France, *Downie 244* (ILL)
Perideridia kelloggii (A. Gray) Mathias	cult. UIUC from seeds obtained from Univ. of California BG, Berkeley, *Downie 635* (ILL), originally from USA, California, Sonoma Co., (*Ornduff et al. s.n.*, UC)
Petroselinum crispum (P. Mill.) A. W. Hill	cult. UIUC, from seeds obtained from Jardin botanique de Caen, France, *Downie 334* (ILL)
Peucedanum terebinthaceum (Fisch. ex Trevis.) Fisch. ex Turez.	cult. UIUC, from seeds obtained from Shanghai BG, China, *Downie 408* (ILL)
Physospermum cornubiense (L.) DC.	cult. Moscow State Univ. BG, Russia, originally from Ukraine, Crimea, Alikat-Bogaz Pass, *Pimenov & Tomkovich s.n.* (MW)
Pimpinella major (L.) Huds.	cult. UIUC, from seeds obtained obtained from Hungarian Academy of Sciences BG, Vácrátót, Hungary, *Downie 92* (ILL)
Pimpinella peregrina L.	cult. UIUC, from seeds obtained from Real Jardín Botánico, Madrid, Spain, *Downie 58* (ILL)
Prionosciadium acuminatum Robins	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-1871)
Prionosciadium turneri Constance & Affolter	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2053), originally from Mexico, Colima, *Turner s.n.* (UC)
Pseudorlaya pumila (L.) Grande	cult. UIUC from seeds obtained from Jardin Botaniques Lisboa, Portugal, *Lee 59* (ILL)
Rhodosciadium argutum (Rose) Mathias & Constance	cult. Univ. of California BG, Berkeley (L. Constance pers. coll. C-2328)
Ridolfia segetum (L.) Moris	Jordan, Wadi Al-Yabis, along R. Jordan, *Lahham & El-Oqlah 12* (Yarmouk Univ. Herbarium)
Scandix pecten-veneris L.	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Downie 27* (ILL)
Selinum candollei DC. (2 accessions)	cult. Univ. of California BG, Berkeley (#89.2000), originally from India, Garhwal Himalaya, Himalaya Mtns., *Pradham s.n.* (UC)
Sium latifolium L.	cult. UIUC, from seeds obtained from Jardin Botanique de Caen, France, *Downie 311* (ILL)
Sium sisarum L.	cult. UIUC, from seed obtained from Real Jardín Botánico, Madrid, Spain, *Downie 53* (ILL)
Smyrnium olusatrum L.	cult. UIUC, from seeds obtained from Quail BGs, California, *Downie 343* (ILL)
Taenidia integerrima (L.) Drude	USA, Illinois, Champaign Co., *Downie 763* (ILL)
Thaspium pinnatifidum (Buckl.) A. Gray	USA, Kentucky, *Downie 810* (ILL)
Tordylium aegyptiacum (L.) Lam. var. *palaestinum* (Zoh.) Zoh.	Jordan, Um-Qais, near Irbid, *Lahham & El-Oqlah 11* (Yarmouk Univ. Herbarium)
Torilis arvensis (Huds.) Link	USA, Illinois, Champaign Co., *Downie 816* (ILL)
Trachyspermum ammi (L.) Sprague ex Turrill	cult. UIUC, from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Downie 14* (ILL)
Turgenia latifolia (L.) Hoffm.	cult. UIUC, from seeds obtained from J.-P. Reduron, Mulhouse, France, *Lee 82* (ILL)
Turgenia latifolia (L.) Hoffm.	Jordan, Eidoon, near Irbid, *Lahham & El-Oqlab 13* (Yarmouk Univ. Herbarium)
Zizia aurea (L.) W. D. J. Koch	cult. UIUC, from seeds obtained from Jardin botanique de Montréal, Canada, *Downie 393* (ILL)

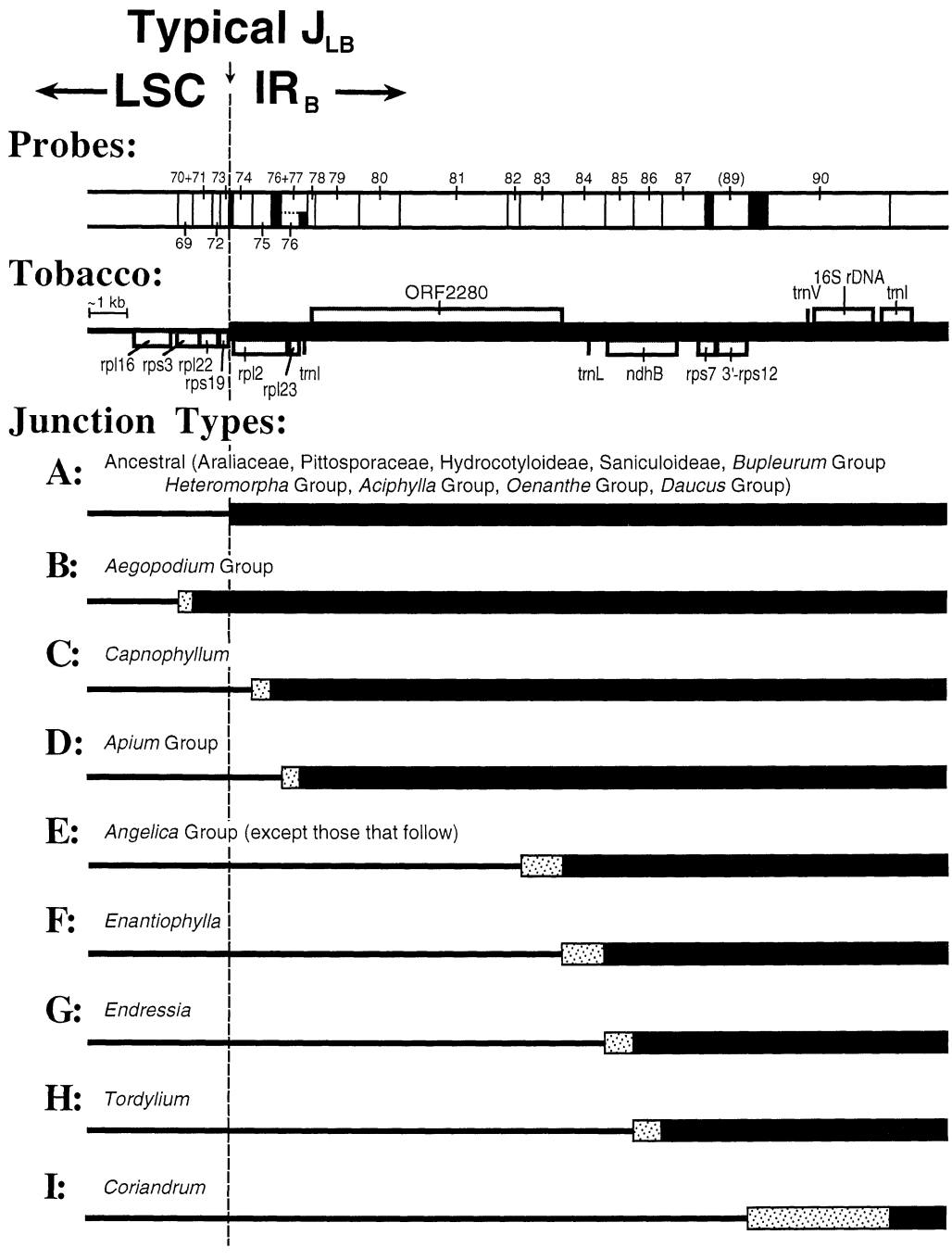

FIG. 2. Variation in position of the nine LSC-IR_B junction (J_{LB}) types (A-I) inferred for 113 representatives of Apiaceae, Araliaceae, and Pittosporaceae (thin line = LSC region; thick line = IR_B region; stippled thick line = area of ambiguity in junction endpoint). The locations of the 19 cloned restriction fragments from tobacco cpDNA used as hybridization probes are indicated relative to its gene map (based on Shinozaki et al. 1986); genes above the line are transcribed from left to right, and those below from right to left. Probes are labeled 69–90 and coincide with those presented in Palmer et al. 1994. Probe 89 was not used, and probes 70 & 71 and 76 & 77 were each combined (probe 76 was also used singly). Blackened boxes in probe map indicate short regions not used as probes. The dashed vertical line represents J_{LB} in tobacco and the vast majority of angiosperms examined to date (Downie and Palmer 1992b).

TABLE 2. Summary and characterization of the nine IR_B-LSC junction types (J_{LB}) in Apiaceae, Araliaceae, and Pittosporaceae cpDNAs. For each junction type (A - I), the direction of IR change (expansion or contraction) and estimated size of the shift and the coding regions potentially affected by it are provided; gene nomenclature follows that of tobacco.

Junction type	Direction of IR change	Shift of J_{LB} relative to tobacco (kb)	Coding regions potentially affected
A	N/A	0	none
B	expansion	+ 1.1	*rps3, rpl22, rps19*
C	contraction	− 0.9	*rpl2*
D	contraction	− 1.6	*rpl2, rpl23*
E	contraction	− 8.5	*rpl2, rpl23, trnI*, ORF 2280
F	contraction	− 9.8	*rpl2, rpl23, trnI*, ORF 2280, *trnL*
G	contraction	−10.8	*rpl2, rpl23, trnI*, ORF 2280, *trnL, ndhB*
H	contraction	−11.5	*rpl2, rpl23, trnI*, ORF 2280, *trnL, ndhB*
I	contraction	−16.1	*rpl2, rpl23, trnI*, ORF 2280, *trnL, ndhB, rps7, 3′-rps12, trnV, 16S rDNA, trnI*

is the smallest among all species mapped, due in part to the ~ 1.1 kb expansion of its IR into previous LSC territory. The progressively smaller genome sizes in those species exhibiting J_{LB} types D—H reflect successively larger IR contractions (Table 3). In *Coriandrum sativum*, one of three species exhibiting the most contracted IR (J_{LB} type I), length changes outside the IR region have offset any change in genome size lost due to the contraction. For example, the IR of *Coriandrum* is ~ 16 kb shorter than the typical IR, but the length of its entire chloroplast genome (~ 150.0 kb) is only 4.5 kb shorter than that of the average typical species; the difference appears to be largely offset by a ~ 5.7 kb insertion of unknown composition into the vicinity of the 16S rRNA gene which is now near the terminus of the IR.

DISCUSSION

Major Lineages within Apiaceae. The most recent treatment of Apiaceae (Pimenov and Leonov 1993) is an adaptation of the century-old system of Drude (1898), criticized for using subtle or poorly defined diagnostic characters (Plunkett et al. 1996b; Downie et al. 1998, 2000). Alternative classifications exist, such as those of Koso-Poljansky (1916) and Cerceau-Larrival (1962), but are rarely used. Drude recognized three subfamilies of Apiaceae (Apioideae, Hydrocotyloideae, and Saniculoideae), dividing each into a series of tribes and subtribes. Systematic investigations based on molecular data have confirmed the monophyly of Apioideae and demonstrated its sister-group relationship to monophyletic Saniculoideae, but have also shown that subfamily Hydrocotyloideae is polyphyletic (e.g., Fig. 3), with some lineages more closely related to Araliaceae than to other Apiaceae (Downie and Katz-Downie 1996; Downie et al. 1998; Plunkett et al. 1996a, 1996b, 1997; Plunkett and Downie 1999; Katz-Downie et al. 2000). These molecular studies confirm that most of Drude's tribes and other reclassifications of the family are unnatural.

Phylogenetic analyses of a variety of molecular characters, such as sequences of chloroplast genes (*rbcL, matK*) and introns (*rpoC1*), nuclear rDNA ITS sequences, and cpDNA restriction sites, yield cladograms that are largely consistent with respect to the major groups resolved (reviewed in Plunkett and Downie 1999). Six major lineages and one paraphyletic group have been recognized within subfamily Apioideae, and are provisionally named the *Aciphylla, Aegopodium, Angelica, Apium, Daucus*, and *Oenanthe* groups (Fig. 3), and the "basal apioid grade" (Plunkett and Downie 1999). The latter has recently been recognized as the *Heteromorpha* and *Bupleurum* groups (Downie et al. 2000). While *Heteromorpha* and *Bupleurum* constitute basally lineages (with the *Heteromorpha* clade sister to all other Apioideae examined), the relationships among the other major groups are not wholly clear. There is, however, strong support for an "apioid superclade," comprising the *Aegopodium, Angelica*, and *Apium* groups (Plunkett and Downie 1999; Fig. 3). Of all eight groups, the *Angelica* group is the largest, and several subclades have been consistently resolved within it (Plunkett and Downie 1999; Downie et al. 2000). Despite the large degree of congruence among different molecular studies, however, the circumscription of the *Aegopodium, Angelica*, and *Apium* clades is not unambiguous, and in studies where the *Apium* group is resolved as monophyletic, it is only weakly supported (Fig. 3; see also

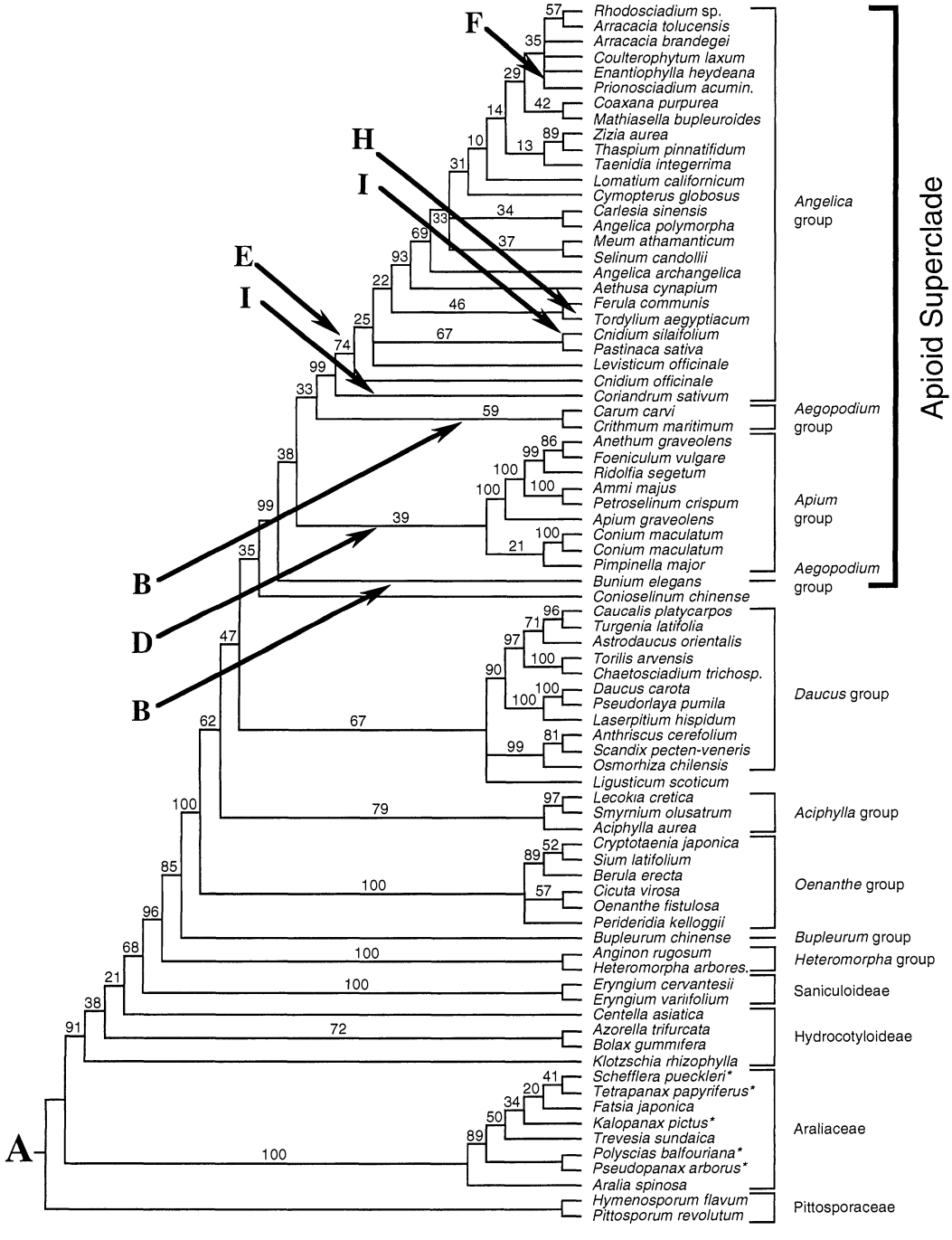

FIG. 3. Strict consensus of 84 minimal length 3038-step trees derived from equally weighted maximum parsimony analysis of cpDNA restriction site data (CI excluding uninformative characters = 0.268; retention index = 0.732; modified from Plunkett and Downie 1999). Bootstrap values for 1000 replicate analyses are shown at the nodes. The distribution of seven of the nine J_{LB} types inferred in this study is indicated; junction types B and I have each occurred in parallel. Five species from Araliaceae included in the restriction site analysis (asterisks) were not surveyed for IR expansion/expansion; many more species were surveyed for the latter, such as those exhibiting junction types C and G, but were

TABLE 3. Estimated sizes (in kb) of the entire chloroplast genome and each of its major structural regions in representative species of Araliaceae, Pittosporaceae, and Apiaceae with regard to its LSC-IR_B junction type. Subfamily Apioideae are divided into seven major clades based on the results of phylogenetic analyses of molecular data (Plunkett and Downie 1999; Downie et al. 1999). Ambiguities in the estimated size of the IR (due to uncertainty of the precise position of J_{LB} within a given probe) are expressed as the average of the minimum and maximum possible sizes, with ranges.

Taxon	Representative species	Junction type	Chloroplast genome structural region			
			LSC	SSC	Each IR	Entire genome
Araliaceae	*Aralia spinosa*	A	85.1	18.8	25.4 ± 0.2	154.7
Pittosporaceae	*Pittosporum revolutum*	A	84.9	20.2	25.1 ± 0.2	155.3
Apiaceae: Hydrocotyloideae	*Centella asiatica*	A	84.0	18.1	25.4 ± 0.2	152.8
Apiaceae: Saniculoideae	*Eryngium varifolium*	A	83.5	19.1	25.5 ± 0.2	153.6
Apiaceae: Apioideae						
Heteromorpha Group	*Heteromorpha arborescens*	A	84.3	19.9	25.5 ± 0.2	155.2
Aciphylla Group	*Aciphylla aurea*	A	84.6	20.1	26.2 ± 0.2	157.0
Oenanthe Group	*Oenanthe fistulosa*	A	83.0	19.4	25.4 ± 0.2	153.1
Daucus Group	*Daucus carota*	A	81.5	19.3	26.0 ± 0.2	152.7
Aegopodium Group	*Carum carvi*	B	79.9	19.3	26.5 ± 0.2	152.2
Apium Group	*Anethum graveolens*	D	86.0	19.4	23.1 ± 0.2	151.5
Angelica Group	*Angelica archangelica*	E	91.2	19.4	16.5 ± 0.5	143.4
	Enantiophylla heydeana	F	92.3	19.2	15.4 ± 0.7	142.3
	Tordylium aegyptiacum	H	95.4	19.3	13.6 ± 0.5	141.8
	Coriandrum sativum	I	113.6	19.4	8.5 ± 1.6	150.0

Plunkett et al. 1996b; Downie et al. 1998; Plunkett and Downie 1999).

Phylogenetic Implications of IR Junction Shifts. To assess the utility of J_{LB} shifts in circumscribing monophyletic groups and to determine the extent of junction mobility during the evolution of subfamily Apioideae, the various J_{LB} types (Table 2; Fig. 1) were superimposed parsimoniously onto a strict consensus tree (Fig. 3) derived from maximum parsimony analysis of cpDNA restriction sites obtained from throughout the entire chloroplast genome (tree length = 3038 steps; CI excluding uninformative characters = 0.268; RI = 0.732; Plunkett and Downie 1999). Five species from Araliaceae included in our earlier analysis were not surveyed for IR expansion/contraction; these are denoted by asterisks in Fig. 3. Conversely, many additional species of Apiaceae were surveyed herein, such as those exhibiting junction types C and G. Because these species were not included in the earlier study, junction types C and G could not be plotted directly on the restriction site tree. Instead, their phylogenetic placements have been inferred from cladograms constructed using other types of molecular data.

Junction type A may be interpreted as ancestral given its distribution in Pittosporaceae and Araliaceae as well as in most other angiosperm groups (Downie and Palmer 1992a,b). Within Apiaceae, this junction type is found in all representatives sampled from subfamilies Hydrocotyloideae and Saniculoideae, and many representatives from subfamily Apioideae, including all members of the basally-branching *Heteromorpha* and *Bupleurum* clades, and in the members of the *Aciphylla, Oenanthe,* and *Daucus* groups (Table 4). Junction type A is also found in *Ligusticum scoticum, Peucedanum terebinthaceum,* and *Physospermum cornubiense,* but the placement of these species is not altogether clear. In analyses of plastid DNA data (e.g., Fig. 3), *L. scoticum* allies with members of the *Daucus* clade, whereas in the ITS studies it is sister to the *Aciphylla* clade. *Physospermum cornubiense* falls near *Heteromorpha* and *Bupleurum* in trees based on plastid DNA data, but is sister to the *Oenanthe* group when ITS se-

←

not included in the former. *Conioselinum chinense* exhibits J_{LB} type A or B. *Ligusticum scoticum* has yet to be assigned to any specific group of umbellifers as it is shows affinity to either the *Daucus* or *Aciphylla* clades depending upon the type of molecular study undertaken.

TABLE 4. Distribution of the nine IR junction types inferred among 113 accessions of Apiaceae, Araliaceae, and Pittosporaceae. Subfamilial treatment of Apiaceae follows Drude (1898), although Hydrocotyloideae are clearly not monophyletic (Plunkett et al. 1996a, 1997). Groups within Apioideae follow Plunkett and Downie (1999) and Downie et al. (1999). Species that could not be unequivocally placed within a group, because of ambiguous junction type or unknown phylogenetic placement, are treated as "uncertain."

Junction type	Taxon
Pittosporaceae	
A	*Hymenosporum flavum*
A	*Pittosporum revolutum*
Araliaceae	
A	*Aralia spinosa*
A	*Fatsia japonica*
A	*Trevesia sundaica*
Apiaceae: Hydrocotyloideae	
A	*Azorella trifurcata*
A	*Bolax gummifera*
A	*Centella asiatica*
A	*Centella erecta*
A	*Didiscus pusilla*
A	*Eremocharis fruticosa*
A	*Klotzschia rhizophylla*
Apiaceae: Saniculoideae	
A	*Astrantia major*
A	*Eryngium cervantesii*
A	*Eryngium varifolium*
A	*Hacquetia epipactis*
A	*Petagnaea saniculifolia*
A	*Sanicula canadensis*
Apiaceae: Apioideae	
Heteromorpha Group	
A	*Anginon rugosum*
A	*Heteromorpha arborescens*
Bupleurum Group	
A	*Bupleurum chinense*
A	*Bupleurum falcatum*
A	*Bupleurum ranunculoides*
A	*Bupleurum rotundifolium*
Aciphylla Group	
A	*Aciphylla aurea*
A	*Anisotome aromatica*
A	*Lecokia cretica*
A	*Smyrnium olusatrum*
Oenanthe Group	
A	*Berula erecta*
A	*Berula thunbergii*
A	*Cicuta virosa*
A	*Cryptotaenia canadensis*
A	*Cryptotaenia japonica*
A	*Oenanthe banatica*
A	*Oenanthe fistulosa*
A	*Perideridia kelloggii*
A	*Sium latifolium*
A	*Sium sisarum*
Daucus Group	
A	*Anthriscus cerefolium*
A	*Astrodaucus orientalis*
A	*Caucalis platycarpos*
A	*Chaetosciadium trichospermum*
A	*Cuminum cyminum*
A	*Daucus carota*
A	*Daucus montanus*
A	*Laserpitium hispidum*
A	*Myrrhis odorata*
A	*Orlaya kochii*
A	*Osmorhiza chilensis*
A	*Pseudorlaya pumila*
A	*Scandix pecten-veneris*
A	*Torilis arvensis*
A	*Turgenia latifolia*
Aegopodium Group	
B	*Aegokeras caespitosa*
B	*Bunium elegans*
B	*Carum alpinum*
B	*Carum carvi*
B	*Crithmum maritimum*
B	*Falcaria vulgaris*
B	*Trachyspermum ammi*
Apium Group	
C	*Capnophyllum dichotomum*
D	*Ammi majus*
D	*Anethum graveolens*
D	*Apium graveolens*
D	*Conium maculatum*
D	*Foeniculum vulgare*
D	*Petroselinum crispum*
D	*Pimpinella major*
D	*Pimpinella peregrina*
D	*Ridolfia segetum*
Angelica Group	
E	*Aethusa cynapium*
E	*Angelica archangelica*
E	*Angelica dahurica*
E	*Angelica decursiva*
E	*Angelica polymorpha*
E	*Arracacia aegopodioides*
E	*Arracacia bracteata*
E	*Arracacia brandegei*
E	*Arracacia pringlei*
E	*Arracacia tolucensis*
E	*Carlesia sinensia*
E	*Chymsydia colchica*
E	*Cnidium officinale*
E	*Coaxana purpurea*
E	*Coulterophytum laxum*
E	*Cymopterus globosus*
E	*Ferula assa-foetida*
E	*Ferula communis*

TABLE 4. Continued.

Junction type	Taxon
E	*Levisticum officinale*
E	*Lomatium californicum*
E	*Mathiasella bupleuroides*
E	*Meum athamanticum*
E	*Notopterygium incisum*
E	*Pastinaca sativa*
E	*Prionosciadium turneri*
E	*Rhodosciadium argutum*
E	*Selinum candollei*
E	*Taenidia integerrima*
E	*Thaspium pinnatifidum*
E	*Zizia aurea*
F	*Enantiophylla heydeana*
F	*Heracleum lanatum*
F	*Prionosciadium acuminatum*
G	*Endressia castellana*
H	*Tordylium aegyptiacum*
I	*Bifora radians*
I	*Cnidium silaifolium*
I	*Coriandrum sativum*
Uncertain	
A or B	*Astomaea sessilifolium*
A or B	*Conioselinum chinense*
A	*Ligusticum scoticum*
A	*Peucedanum terebinthaceum*
A	*Physospermum cornubiense*

quences are compared (Downie et al. 1998). Molecular data from *Peucedanum terebinthaceum* are available only for ITS sequence, which suggest that this species is allied to *L. scoticum* (S. Downie, unpubl. data).

Junction type B, representing a 1.1 kb expansion of the IR relative to J_{LB} type A, is found in all taxa sampled from the *Aegopodium* group. Not all studies, however, support this group as monophyletic. For example, the analysis of *rpoC1* intron sequences places *Crithmum* and *Trachyspermum ammi* (the *Crithmum* clade) sister to the *Angelica* group and away from the clade of *Aegokeras* (syn. *Olymposciadium*), *Aegopodium, Carum,* and *Falcaria* (Downie et al. 1998). Furthermore, while ITS studies place *Bunium elegans* alongside *Crithmum* in the *Aegopodium* clade (Downie et al. 2000), the analysis of cpDNA restriction sites treats *Bunium* as an isolated lineage away from this group (Fig. 3). On the basis of these studies, junction type B can be inferred to have occurred either singly during the evolution of the subfamily or in parallel twice.

All members of the *Apium* group are characterized by J_{LB} type D (Fig. 3), with the exception of *Capnophyllum* which exhibits J_{LB} type C (not shown). The genera *Ammi, Anethum, Apium, Foeniculum, Petroselinum,* and *Ridolfia* form a strongly supported clade in all analyses of molecular data to date (the *Apium* clade sensu stricto), but their relationship to *Capnophyllum, Conium,* and *Pimpinella* is weak. Indeed, phylogenetic analysis of ITS sequences (Downie et al. 1998; Katz-Downie et al. 1999) shows that *Capnophyllum, Conium,* and *Pimpinella* each comprise separate lineages at the base of the *Angelica* clade. Consequently, at least three independent derivations can be postulated for junction type D. On the other hand, phylogenetic analyses of *matK* and *rpoC1* intron sequences (Plunkett et al. 1996b; Downie et al. 1998) and cpDNA restriction sites (Fig. 3; Plunkett and Downie 1999) support the union of *Pimpinella* with the *Apium* clade sensu stricto (the last two studies also add *Conium*), suggesting that J_{LB} type D is synapomorphic. In analyses based on combined *rpoC1*-intron and *rpl16*-intron data (Downie et al. 2000), *Capnophyllum* is placed in the *Apium* clade (specifically, sister to the *Apium* clade s. str.), suggesting that J_{LB} type C evolved from an ancestor possessing J_{LB} type D. In this scenario, a slight expansion of the IR is invoked.

Junction type E characterizes 30 of the 38 species sampled from the *Angelica* group; the remaining species are characterized by junction types F–I. Junction types F and I were each found in three species (type F in *Enantiophylla heydeana, Heracleum lanatum,* and *Prionosciadium acuminatum,* and type I in *Bifora radians, Cnidium silaifolium,* and *Coriandrum sativum*), whereas types G and H were both restricted to a single species (*Endressia castellana* and *Tordylium aegyptiacum,* respectively). The meso-American genera *Enantiophylla* and *Prionosciadium* unite as monophyletic in almost all analyses to date (e.g., Downie and Katz-Downie 1996; Downie et al. 1998; Plunkett and Downie 1999) but their putative union with *Heracleum* on the basis of shared junction type (type F) is surprising given the results from phylogenetic studies. As such, it appears that J_{LB} type F may have originated at least twice during the evolution of the group. Similarly, while the relationship between *Bifora radians* and *Coriandrum sativum* is well-supported in many studies, these two species have yet to be associated with *Cnidium silaifolium.* As a consequence, junction type I may be homoplastic as well.

More broadly, all shifts in J_{LB} position, including types C and G, are restricted to the apioid superclade (i.e., the *Aegopodium, Apium,* and *Angelica*

groups, collectively) (Fig. 3; Plunkett and Downie 1999). This finding may have important implications for the evolution of these structural changes, and the proclivity for members of this clade to exhibit a diversity of J_{LB} types evokes some common mechanism that may have originated in the immediate common ancestor of the group. Moreover, the exclusion of both *Astomaea* and *Conioselinum* from the apioid superclade in phylogenetic studies (Downie et al. 1998; S. Downie, unpubl. data) suggests that these species (whose junction types could not be determined unambiguously) possess J_{LB} type A since junction shifts are found only within the apioid superclade.

In mapping the junction types parsimoniously onto the phylogenetic tree based on restriction site data (Fig. 3), a minimum of two expansions (both J_{LB} type B) and six contractions (J_{LB} types D, E, F, H, and two independent origins of type I) of the IR can be inferred. The presence of additional contractions in *Capnophyllum* (J_{LB} type C) and *Endressia* (J_{LB} type G), taxa not included in this tree, brings the number of contraction events to eight. An alternative hypothesis (based on the cladograms of Downie et al. 2000) suggests a small expansion leading to *Capnophyllum,* but the placement of this species within the *Apium* clade needs further clarification. Considering all of the available molecular data and the trees inferred from them, many more instances of homoplasy could be inferred, particularly with regard to the distribution of IR junction types D, F, and I. However, lacking a single, well-resolved phylogenetic hypothesis (based on a combination of all data sets), it is not yet possible to rigorously evaluate the extent of J_{LB} mobility and homoplasy. It does seem, however, that junction types F, H, and I (and likely type G based on the position of *Endressia* in Downie et al. 1998) originated from a common ancestor possessing junction type E (Fig. 3) and that several presumably identical junction types have occurred in parallel. In other studies, increased sampling places *Coriandrum* well within the *Angelica* clade, providing further support for the independent derivations of type I junctions from a type E ancestor (Downie et al. 1998). Reversals in junction type are not apparent.

Although shared structural mutations can provide strong evidence of common ancestry, it is apparent that similar rearrangements, such as intron losses and inversions, can occur independently (Downie et al. 1991, 1996; Doyle et al. 1995). Within the limits of detection of our mapping studies, it now appears that specific expansion and contraction events of the IR are not immune to homoplasy. Similar results have been reported for species of *Ranunculus* where the IR has contracted 200–300 bp at least eight times and in one instance a reversal has been evoked to explain their phylogenetic distribution (Johansson 1998). While the hybridization probes used in our investigation are smaller than those typically used in comparative restriction site mapping studies, ambiguity remains in assessing the precise terminus of the IR in the absence of DNA sequence data. Sequence analysis reveals that small differences in IR endpoints (<100 bp) are common among closely related species (Goulding et al. 1996) and it is not unrealistic to presume that such differences exist in Apioideae. Further study may reveal that the IR endpoints in *Coriandrum* and *Cnidium* are not identical, and this may also prove to be the case in putatively unrelated *Enantiophylla* and *Heracleum.* No doubt other J_{LB} positional variants will be found and the positions of existing ones refined as DNA sequence data become available.

IR Expansion/Contraction in Angiosperms. A previous study on the evolution of chloroplast genome structural organization yielded data on the position of J_{LB} in many major lineages of flowering plants (Downie and Palmer 1992a,b, unpubl. data). Restriction site maps for four restriction enzymes (*Bam*HI, *Hin*dIII, *Bgl*II, *Eco*RV) were constructed by hybridizing 106 tobacco cpDNA probes (including the 19 used in this study; Fig. 2) to filter-blots containing digests of 113 species of angiosperms from nine monocot and 45 dicot families. The latter included representation of all six subclasses of dicots (Cronquist 1981) including 34 families and 85 species from the large subclass Asteridae. With the exception of the parasitic asterid genera *Conopholis* (Orobanchaceae) and *Striga* (Scrophulariaceae) and four species of Campanulaceae, where either the IR was lost or rearrangements within or near the IR made the position of J_{LB} difficult to ascertain, the results indicated that the vast majority of angiosperms examined possess a J_{LB} matching that of tobacco (that is, lying within gene *rps19* in IR_B), a finding consistent with the highly conserved nature of cpDNA structure. Nineteen species exhibited shifts in J_{LB} position relative to tobacco (Table 5), ranging from an expansion of ~ 2.6 kb in *Kolkwitzia* (Caprifoliaceae) to a contraction of ~ 3.5 kb in Myoporaceae and Loganiaceae. In *Kolkwitzia,* J_{LB} lies near the 5′ end of gene *rpl16* (Fig. 2). Some junction shifts are synapomorphic (such as the 2.0 kb contraction in all members of Convolvulaceae and Cus-

TABLE 5. Species exhibiting variation in position of J_{LB} relative to the tobacco J_{LB} (Downie and Palmer 1992b, and unpubl. data). Members of the apioid superclade and other non-umbellifer species cited in text are omitted.

Family	Species	Approx. location of J_{LB} (see Fig. 2) direction ("+" = expansion; "−" = contraction), and size (kb) of shift relative to tobacco J_{LB}
Caprifoliaceae:	*Kolkwitzia amabilis*	*rpl16* (+2.6)
Boraginaceae:	*Borago officinalis*	probe 69 (+1.4)
Gentianaceae:	*Exacum affine*	probe 69 (+1.4)
Dipsacaceae:	*Cephalaria leucantha*	probe 70 (+1.0)
	Dipsacus sp.	probe 70 (+1.0)
Callitrichaceae:	*Callitriche heterophylla*	probe 70 (+1.0)
Commelinaceae:	*Commelina* sp.	probe 71 (+0.6)
Valerianaceae:	*Valeriana* sp.	probe 76 (−1.8)
Plumbaginaceae:	*Limonium gmelinii*	probe 76 (−1.8)
Caprifoliaceae:	*Lonicera subsessilis*	probe 76 (−1.8)
	Weigela hortensis	probe 76 (−1.8)
Convolvulaceae:	*Calonyction aculeatum*	probe 77 (−2.0)
	Convolvulus tricolor	probe 77 (−2.0)
	Ipomoea pes-caprae	probe 77 (−2.0)
Cuscutaceae:	*Cuscuta* sp.	probe 77 (−2.0)
Myoporaceae:	*Bontia daphnoides*	probe 79 (−3.5)
	Eremophila maculata	probe 79 (−3.5)
	Myoporum sandwicense	probe 79 (−3.5)
Loganiaceae:	*Gelsemium sempervirens*	probe 79 (−3.5)

cutaceae) whereas others are homoplastic (such as the 1.8 kb contraction in Valerianaceae, Plumbaginaceae, and two of three species of Caprifoliaceae). These data corroborate the results of Goulding et al. (1996) and others, and our own studies of Apiaceae, in showing that IR/LSC boundary positions are not static but can indeed expand and contract moderately (1–4 kb) during angiosperm evolution.

Molecular Evolutionary Implications. Phylogenetic studies have suggested that the IR has both expanded and contracted during the evolution of subfamily Apioideae. At least one expansion and seven contraction events can be postulated within the limits of our experiments, with several of these occurring in parallel when considered in a phylogenetic context. While contractions can be explained by the deletion of DNA from within one copy of the IR (and, for several apioid species, at least two contraction events must be evoked to explain present J_{LB} positions), explanations for IR expansion are more complex. No consensus exists as to the mechanisms responsible for these changes, but many theories invoke homologous recombination between repeated regions. Small dispersed repeat elements have been documented for many species with rearranged chloroplast genomes. For example, to explain a 41-kb expansion of the IR in *Chlamydomonas reinhardtii* Dangeard, Palmer et al. (1985) proposed a mechanism that involved the pairing of short repeat elements located outside the IR and also pairing of the IR itself, followed by copy-correctional duplication of the intervening region. Other taxa where rearrangements have been associated with dispersed repeats include *Pelargonium* × *hortorum* (Palmer et al. 1987a), *Pseudotsuga menziesii* (Mirbel) Franco (Strauss et al. 1988), *Trifolium subterraneum* L. (Milligan et al. 1989), *Epifagus virginiana* (L.) Barton (Wolfe et al. 1992), *Anemone* (Hoot and Palmer 1994), maize (Maier et al. 1995), and *Trachelium caeruleum* L. (Cosner et al. 1997). It has also been noted that rearrangement endpoints and/or dispersed repeats are frequently found adjacent to tRNA genes (e.g., Howe et al. 1988; Hiratsuka et al. 1989; Palmer 1991; Hoot and Palmer 1994), but the relationship of tRNA genes with repeated segments and/or rearrangements is poorly understood. Short dispersed repeat elements are considered rare in the chloroplast genome (Palmer 1985a), and explanations for their origins have evoked such phenomenon as transposable elements (Milligan et al. 1989; Zhou et al. 1988). Another explanation for their origin involves replication slippage and mispairing [especially at A-T rich or poly(A) tracts], a theory used to explain the origin of short repeats found in the plastid genomes of *Epifagus virginiana* (Wolfe et al. 1992) and *Oen-*

othera (Wolfson et al. 1991; Sears et al. 1996). Lastly, Goulding et al. (1996) invoked gene conversion to explain the very small shifts in IR endpoints of several species of *Nicotiana* and related dicots, but suggested a more elaborate mechanism of double-stranded breakage followed by DNA repair and recombination at poly(A) tracts to account for the 12 kb expansion of the IR in *N. acuminata.*

It is difficult to pinpoint the precise mechanism responsible for the numerous and variable junction shifts seen in Apioideae since sequence data are lacking. Some general inferences, however, can be made. First, several junction shifts are adjacent to tRNA genes: junction types E and F are adjacent to *trnL*; type D is adjacent to *trnI*; and type I is adjacent to either *trnV* or *trnI* depending on where the terminus is located (Fig. 2). If short repeats or poly(A) tracts are located in or adjacent to these regions (as they are in several other plant groups exhibiting major rearrangements), then intramolecular recombination between them may explain the structural mutations found in this subfamily. Second, because all junction shifts are restricted to a single clade, they may not represent entirely independent events. A single mutation in the common ancestor of the apioid superclade, such as the insertion of a repeated segment or some initial expansion/contraction event, may have set off a series of subsequent rearrangements in descendent lineages. Such a scenario is evident in several other plant groups with highly-rearranged cpDNAs, where a single mutation has been hypothesized to have initiated a series of additional changes (e.g., Palmer and Thompson 1982; Palmer et al. 1987b, 1988).

The flowering plant family Apiaceae comprises some 455 genera and 3,500 species (Pimenov and Leonov 1993) of which we have examined only 75 genera and 108 species. The screening of junction types in hitherto unexamined species may provide a quick means of ascertaining their broad phylogenetic placement or to confirm, at least, their membership in the apioid superclade. Similarly, the distribution of junction types may help decide among incongruent trees, although the potential for homoplasy can confound issues of relationship based solely on these rearrangement characters. The large size and frequency of LSC-IR junction shifts in Apioideae are unprecedented among angiosperms, and suggests that the subfamily represents a model system for which to study the mechanisms leading to large-scale expansions and contractions of the IR. To this end, we have initiated sequencing through these LSC-IR endpoints to reveal the underlying mechanisms responsible for these changes. This information will also enable us to identify homologous J_{LB} types.

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