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Characterization and phylogenetic distribution of a chloroplast DNA rearrangement in the *Berberidaceae*

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Abstract: Restriction site maps and a clone bank of chloroplast DNA (cpDNA) of *Mahonia higginsae* (MUNZ) AHRENDT (*Berberidaceae*) were constructed. The size of *Mahonia* cpDNA was about 167 kb. Precise mapping using gene probes revealed that cpDNA of *M. higginsae* has an inverted repeat (IR) 11.5 kb larger than the tobacco IR. The expansion of the IR into the large single copy region has resulted in the duplication of at least ten genes including *psb*B. The phylogenetic distribution of the expanded IR was examined in twenty-five species of *Berberis* and *Mahonia*, twenty species representing the fifteen remaining genera of the *Berberidaceae*, and four species from four allied families. Our survey indicates that only the species of the closely related genera *Berberis* and *Mahonia* share the 11.5 kb expansion of IR. This result supports their close phylogenetic relationship, which has been suggested previously by chromosomal, morphological, and serological data.

The chloroplast genome of land plants shows a high degree of conservation in size, gene arrangement, and gene content (PALMER & STEIN 1986, SUGIURA 1989, PALMER 1991). One of the most conservative structural features in chloroplast DNA (cpDNA) is the presence of large inverted repeats (IR) separated by small and large single copy regions. Excluding some legumes (LAVIN & al. 1990) and conifers (STRAUSS & al. 1988, LIDHOLM & al. 1990, RAUBESON & JANSEN 1992) which lack the IR, most seed plants possess an IR that ranges in size from 20 to 30 kilobase pairs (kb) (PALMER & STEIN 1986, DOWNIE & PALMER 1991) except in a few extreme cases (PALMER & al. 1987, DEPAMPHILIS & PALMER 1989).

The *Berberidaceae* are a heterogeneous family of three well-known ornamental woody genera (*Berberis, Mahonia*, and *Nandina*) and 14 small herbaceous genera (Table 1). The family belongs to the *Ranunculales* and is closely allied to the *Ranunculaceae, Hydrastidaceae, Lardizabalaceae*, and *Menispermaceae* (LOCONTE & ESTES 1989). The long evolutionary history and high degree of morphological divergence among genera of the *Berberidaceae* provide this family with many compelling systematic questions (LOCONTE & ESTES 1989, MEACHAM 1980). Although the family has been examined extensively using a wide diversity of approaches, including morphology (CHAPMAN 1936, NOWICKE & SKVARLA 1981, TERABAYASHI 1985) and serology (JENSEN 1973), much of the discordance concerning phylogenetic

Table 1. Collecting data for the taxa used in this study. Accession numbers are used as voucher information for plants collected at *AA* Arnold Arboretum, *RSABG* Rancho Santa Ana Botanical Garden, *NYBG* New York Botanical Garden, *HUBG* Hokkaido University Botanical Garden, *KEW* Royal Botanic Gardens Kew, and Royal Botanic Garden Edinburgh

Species	Source/Voucher	Presence of expanded IR
Berberidaceae		
Berberis brachypoda MAXIM.	AA, 1065-69	Yes
B. amurensis RUPR.	AA, 1449–77	Yes
B. candidula SCHNEID.	AA, 21131	Yes
B. circumserrata SCHNEID.	AA, 1098-65	Yes
B. conferta KUNTH	PANERO 2937 (TEX)	Yes
B. dielsiana FEDDE	AA, 7570	Yes
B. gagnepainii SCHNEID.	AA, 21133	Yes
B. gilgiana SCHNEID.	AA, 7283	Yes
B. julianae SCHNEID.	AA, 7178	Yes
B. thunbergii DC.	AA, 627-52	Yes
B. vernae SCHNEID.	AA, 12763	Yes
B. vulgaris L.	AA, 12770	Yes
B. warszewiczii HIERON.	PANERO 2946 (TEX)	Yes
B. wilsonae HEMSL.	AA, 65574	Yes
B. zabeliana SCHNEID.	AA, 758-84	Yes
Mahonia amplectens EASTWOOD	RSABG	Yes
M. aguifolium (PURSH) NUTT.	AA, 15193	Yes
M. higginsae (MUNZ) AHRENDT	YDK 90-1 (TEX)	Yes
M. japonica (THUNB.) DC.	Edinburgh, 751273	Yes
M. nervosa (PURSH) NUTT.	BALDWIN 746 (TEX)	Yes
M. nevinii (GRAY) FEDDE	RSABG	Yes
M. repens G. DON	Edinburgh, 800151	Yes
M. pinnata (LAG.) FEDDE	AA, 142-55	Yes
M. piperiana ABRAMS	AA, 393-57	Yes
M. trifoliata (MORIC.) FEDDE	YDK 93-1 (TEX)	Yes
Achlys triphylla DC.	YDK 92-24 (TEX)	No
A. japonica MAXIM.	HUBG, 11797	No
Bongardia chrysogonum SPACH	KEW, 73	No
Caulophyllum robustum MAXIM.	YDK 92-5 (TEX)	No
C. thalictroides MICHX	MEHRHOFF 12985 (CONN)	No
Dysosma pleiantha WOODSON	J. WEN 1390 (A)	No
Diphylleia cymosa MICHX	J. WEN 977 (A)	No
D. Grayi F. SCHMIDT	Edinburgh, 821338	No
Epimedium korenum NAKAI	YDK 92-8 (TEX)	No
Gymnospermium microrrhynchum TAKHT.	YDK 92-13 (TEX)	No
Jeffersonia diphylla PERS.	YDK 91-1 (TEX)	No
Leontice eversmannii BUNGE	KEW, 3520	No
Nandina domestica THUNB.	YDK 94-1 (TEX)	No
Plagiorhegma dubia MAXIM.	YDK 92-37 (TEX)	No
P. peltatum L.	YDK 92-1 (TEX)	No
Ranzania japonica ITO	Edinburgh, 741910	No
Sinopodophyllum hexandrum YING	Edinburgh, 841879	No

Table 1 (continued)

Species	Source/Voucher	Presence of expanded IR
Vancouveria hexandra C. MORR. & DECNE.	YDK 92-28 (TEX)	No
V. planipetala CALLONI	YDK 92-31 (TEX)	No
Ranunculaceae		
Glaucidium palmatum SIBOLD & ZUCC.	AA	No
Hydrastidaceae		
Hydrastis canadensis L.	YDK 91-2 (TEX)	No
Lardizabalacelae		
Akebia quinata DECNE	JOHANSSON 166 (CONN)	No
Menispermaceae		
Cocculus carolinus DC.	YDK 93-2 (TEX)	No

relationships in the *Berberidaceae* still exists due to extensive convergent and parallel evolution of the morphological characters (MEACHAM 1980). New data, especially from rigorous molecular studies, are needed to test competing hypotheses about intergeneric relationships and character evolution in the family.

In this study we characterized a structural change in the cpDNA of *Mahonia higginsae* (MUNZ) AHRENDT and determined the phylogenetic distribution of the structural change in the *Berberidaceae*. We also have constructed restriction site maps of the *Mahonia* cpDNA and cloned probes for use in future restriction site studies in the family.

Material and methods

Chloroplast DNA of *Mahonia higginsae* was isolated by the sucrose gradient technique (PALMER 1986). Leaf material of *Mahonia* was obtained from the Rancho Santa Ana Botanical Garden in California. Total DNAs of 49 species including outgroups of the *Berberidaceae* (Table 1) have been isolated according to the CTAB method of DOYLE & DOYLE (1987), followed by further purification in cesium chloride-ethidium bromide gradients. Restriction endonuclease digestions, agarose gel electrophoresis, bidirectional transfer of DNA fragments from agarose gels to Zetabind (AMF CUNO) nylon filters, labeling of recombinant plasmids by nick-translation, filter hybridizations, and autoradiography were performed as described (PALMER 1986). Hybridizations were performed at 58 °C for 16 h. A restriction site map of *M. higginsae* for 10 restriction endonucleases has been constructed using single and double digestions and the overlap hybridization strategy described by PALMER (1986). The *Nicotiana tabacum* L. cpDNA clone bank (SUGIURA & al. 1986) including 43 subclones (OLMSTEAD & PALMER 1992) and *Mahonia higginsae* cpDNA clones were used. For the precise characterization of the extended inverted repeat, small tobacco gene probes (provided by J. D. PALMER) were used. The *M. higginsae* cpDNA was cloned using the restriction enzyme *Hind*III, which generated 26 restriction fragments ranging in size from 0.3–11.3 kb. Five mg of DNAse I-purified *M. higginsae* cpDNA was digested with *Hind*III and ligated with 50 ng of *Hind*III digested pUC 19. The ligation mixture was used to transform *E. coli* strain DH 5α and recombinant white colonies were selected on ampicillin/X-gal plates. About 600 recombinant white colonies were screened by the alkaline lysis plasmid miniprep method (MANIATIS & al. 1982).

Results

Physical structure of the *Mahonia higginsae* chloroplast DNA. The shotgun strategy for cloning *Mahonia higginsae* cpDNA yielded clones containing 14 of the 26 *Hind*III restriction fragments (Fig. 1), which covers about 70% of the chloroplast genome.

Chloroplast DNA from *M. higginsae* was digested with 10 restriction endonucleases chosen for physical mapping. Twenty to sixty-seven restriction fragments were generated depending on restriction enzymes. Summation of the restriction fragment sizes yields a genome size of approximately 167 kb, about 11.5 kb larger than that of tobacco cpDNA. The relative position of the restriction sites was determined by double digestion with *Sst* I. Successive overlapping hybridization of the filters to 43 tobacco subclones revealed that chloroplast genome of *M. higginsae* is collinear with that of *Nicotiana tabacum*. The restriction site map of the *M. higginsae* chloroplast genome is shown in Fig. 1. The most noticeable feature is the presence of the expanded IR, which is 11.5 kb longer than the tobacco IR (ca. 25.3 kb). The expansion has occurred by spreading of the IR into large single copy region, unaccompanied by any change in sequence complexity. Thus, the large single copy region hybridizing to tobacco subclones 25, 26, 27, and 28 (Fig. 1) is nested within the IR in *Mahonia* cpDNA. Detailed mapping using tobacco gene probes revealed that the expansion of the IR resulted in duplication of 11 genes located outside of the tobacco IRb including *psb* B, ORF 34, *psb* N, *psb* H, *pet* B, *pet* D, *rpo* A, *rps* 8, *rpl* 16, *rps* 3, and *rps* 19 (only four of them are shown in Fig. 2). These genes are thought to constitute two operons, the *psb* B and *rpl* 23 operons (DOWNIE & PALMER 1991).

Phylogenetic distribution of the expanded inverted repeat. The phylogenetic distribution of the expanded inverted repeat was examined in 25 species of *Berberis* and *Mahonia* from 13 sections (AHRENDT 1961). We also examined a representative species from the 15 remaining genera of *Berberidaceae* and five species from five putatively related families (Table 1). Total DNAs were digested using four restriction endonucleases (*Bam* HI, *Eco* RV, *Hind* III, and *Sst* I) and filters were hybridized to seven tobacco subclones (2, 3, 24–28; see Fig. 1) to determine the presence of the expanded IR. Based on local mapping we hypothesized that presence of two flanking fragments hybridizing to tobacco subclone 25 and overlap of one of these fragments to subclone 2 is the evidence of the 11.5 kb expansion of the IR (Fig. 2). For example, in *M. higginsae* probe 25 hybridizes to two flanking *Sst* I fragments of 13.0 and 15.3 kb and probe 2 only hybridizes to the 15.3 kb fragment. The results (Table 1) of our flanking fragment strategy show that only the species of *Mahonia* and *Berberis* share the 11.5 kb expanded IR.

Discussion

Accumulation of the information from the fine-scale physical mapping studies from various cpDNAs revealed that the contraction and expansion of IR is relatively common (PALMER & STEIN 1986, PALMER & al. 1987, HASEBE & IWATSUKI 1990). Along with gene insertion (PALMER & al. 1987), the expansion of IR into adjacent single-copy regions is known as one of major causes of the size increase in cpDNA (DOWNIE & PALMER 1991). The best known example is *Pelargonium* × *hortorum* (PALMER & al. 1987) in which the unusually large size of the cpDNA (217 kb) is

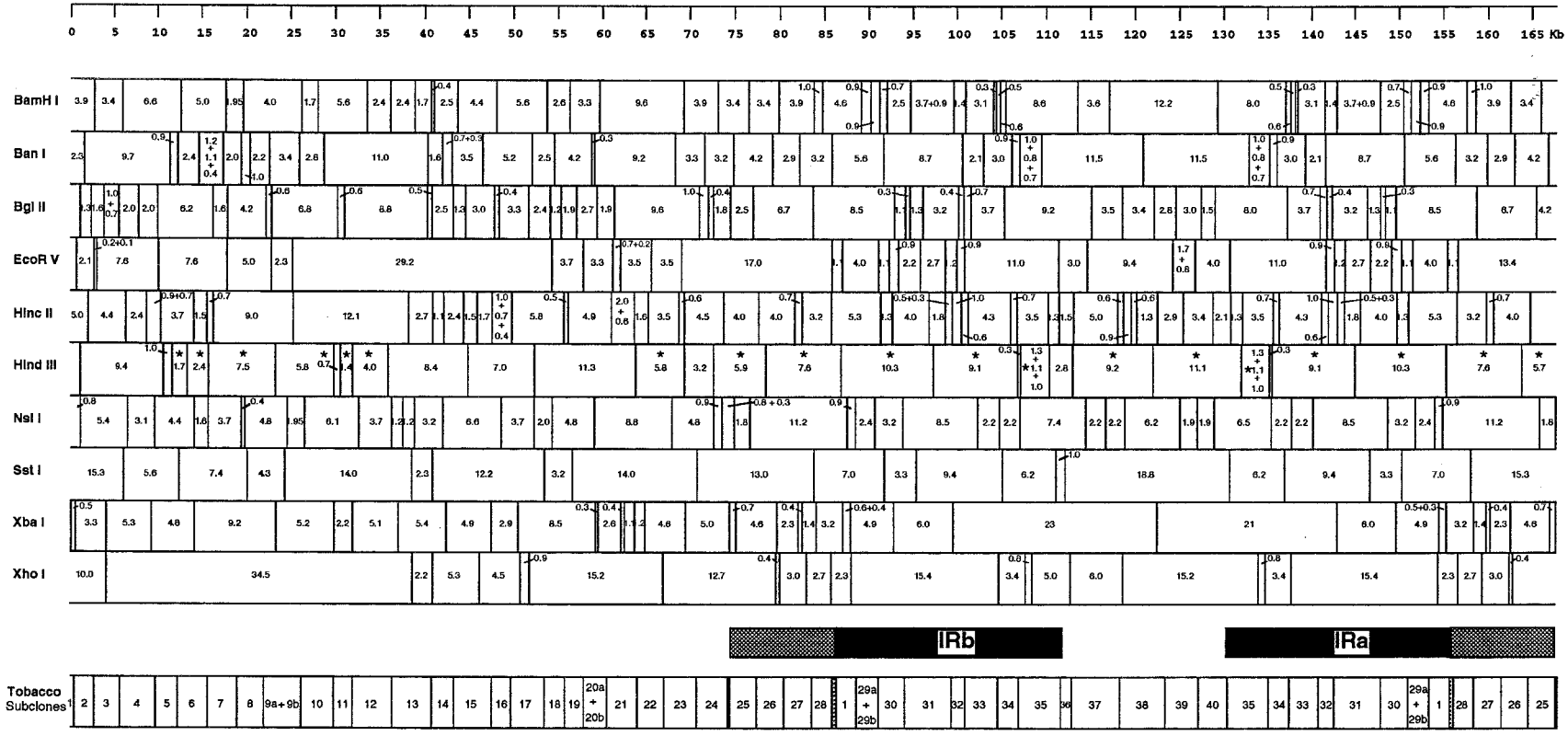

Fig. 1. Restriction site maps of *Mahonia higginsae* cpDNA. The circular maps have been linearized to compare with tobacco map. Numbers in tobacco map indicate a serial subclone number by OLMSTEAD & PALMER (1992). The tobacco subclone map has been modified to account for the expansion of the IR in *M. higginsae* cpDNA. Dark bars indicate extent of the IR (a and b) of tobacco cpDNA. Shaded bars represent the expansion of IR in *Mahonia* cpDNA. Numbers in *M. higginsae* maps denote the size of restriction fragments in kilobases. Asterisks in *Hind* III map indicate cloned restriction fragments

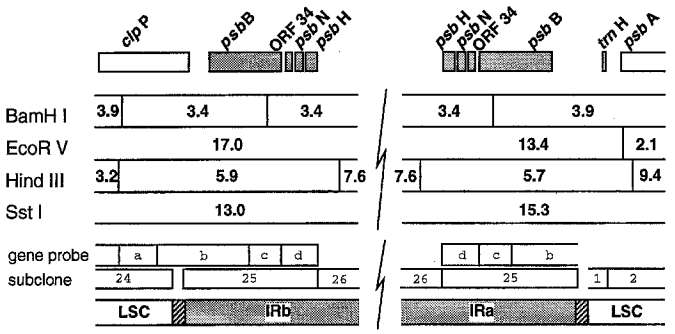

Fig. 2. Localized restriction site maps of *Mahonia higginsae* cpDNA depicting experimental strategy for detecting 11.5 kb expansion of the IR (size in kb). Only the junctions between Large Single Copy (*LSC*) region and IR (a and b) are shown for simplifications (see Fig. 1 for detail). Tobacco gene probes a (841 bp; *clp* P intron 2, *clp* P exon 2, *clp* P intron 1), b (2086 bp; *clp* P intron 1, *clp* P exon 1, 5′ *psb* B), c (723 bp; 3′ *psb* B), and d (830 bp; ORF 34, *psb* N, *psb* H) were used to determine the extent of expansion. Hatched boxes indicate maximum and minimum extent of the expanded IR determined by 0.7 kb *Xba* I restriction fragment (see Fig. 1) and tobacco subclone 24, respectively

achieved mostly by the 50 kb expansion of the IR. Likewise, since we could not detect any gene insertion, the size increase of *Mahonia* cpDNA is attributed to the expansion of IR and its associated gene duplication.

Because of the conservative nature in the size of IR, length variations have been used to define monophyletic groups in the *Ranunculaceae* (JOHANSSON & JANSEN 1993) and the *Oleaceae* (K.-J. KIM & R. JANSEN, unpubl.). The sharing of the 11.5 kb expansion of the IR among the members of *Berberis* and *Mahonia* supports their close phylogenetic relationship. Although *Mahonia* differs from *Berberis* in lacking thorns and having compound leaves, these two arborescent genera have been considered to be very closely related to each other and even congeneric (DERMEN 1931, JENSEN 1973, LOCONTE & ESTES 1989). Several systematic studies based on chromosome number (DERMEN 1931), wood anatomy (SHEN 1954), floral anatomy (TERABAYASHI 1978), pollen (NOWICKE & SKVARLA 1981), seedling morphology (TERABAYASHI 1987), and serology (JENSEN 1973) reported no significant difference between *Berberis* and *Mahonia*. Based on embryological study, however, SASTRI (1969) concluded that the two genera differ in the presence and absence of a rudimentary aril, and in the nature of the antipodals, synergids, and endothecium. AHRENDT (1961) postulated that *Berberis* is derived from *Mahonia* by reducing the compound leaf into a thorn and simple leaf. However, additional molecular data are needed to determine the monophyly and circumscription of these genera as well as the phylogenetic relationship of these distinct woody genera to the remaining members of *Berberidaceae*.

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