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CHLOROPLAST DNA PHYLOGENY, RETICULATE EVOLUTION, AND BIOGEOGRAPHY OF *PAEONIA* (PAEONIACEAE)[1]

TAO SANG,[2] DANIEL J. CRAWFORD, AND TOD F. STUESSY[3]

Department of Plant Biology, Ohio State University, Columbus, Ohio 43210

The coding region of the *mat*K gene and two intergenic spacers, *psb*A-*trn*H and *trn*L(UAA)-*trn*F(GAA), of cpDNA were sequenced to study phylogenetic relationships of 32 *Paeonia* species. In the *psb*A-*trn*H intergenic spacer, short sequences bordered by long inverted repeats have undergone inversions that are often homoplasious mutations. Insertions/deletions found in the two intergenic spacers, mostly resulting from slipped-strand mispairing, provided relatively reliable phylogenetic information. The *mat*K coding region, evolving more rapidly than the *trn*L-*trn*F spacer and more slowly than the *psb*A-*trn*H spacer, produced the best resolved phylogenetic tree. The *mat*K phylogeny was compared with the phylogeny obtained from sequences of internal transcribed spacers (ITS) of nuclear ribosomal DNA. A refined hypothesis of species phylogeny of section *Paeonia* was proposed by considering the discordance between the nuclear and cpDNA phylogenies to be results of hybrid speciation followed by inheritance of cpDNA of one parent and fixation of ITS sequences of another parent. The Eurasian and western North American disjunct distribution of the genus may have resulted from interrruption of the continuous distribution of ancestral populations of extant peony species across the Bering land bridge during the Miocene. Pleistocene glaciation may have played an important role in triggering extensive reticulate evolution within section *Paeonia* and shifting distributional ranges of both parental and hybrid species.

Key words: biogeography; chloroplast DNA; hybridization; *mat*K; *Paeonia*; *psb*A–*trn*H spacer; reticulate evolution; *trn*L–*trn*F spacer.

Paeonia comprises ~35 species of shrubs and perennial herbs distributed widely in five disjunct areas in the northern hemisphere: eastern Asia, central Asia, the western Himalayas, the Mediterranean region, and Pacific North America (Stern, 1946; Pan, 1979; Tzanoudakis, 1983; Pei, 1993). The genus is systematically isolated, having been placed in the unigeneric family *Paeonia*ceae, which has either been placed by itself or together with Glaucidiaceae in order Paeoniales (Takhtajan, 1969, 1987; Thorne, 1992). Because of their great ornamental and medicinal value, peonies have been known as ''king of flowers'' in China and ''queen of herbs'' in Greece for > 1000 yr (Gambrill, 1988).

Three sections are recognized within *Paeonia* (Stern, 1946). Section *Oneapia*, endemic to Pacific North America, comprises two herbaceous species with conspicuous staminodial disks and small fleshy concave petals. Section *Moutan* with six species, occurring in central and western China, was divided into two subsections, *Delavayanae* and *Vaginatae*. They are shrubs with conspicuous staminodial disks and large spreading petals. Section *Paeonia* (''*Paeon*''), which includes the type species *P. officinalis*, was also divided into two subsections, *Foliolatae* and *Paeonia* (''*Dissectifoliae*''), distributed disjunctly in eastern Asia, central Asia, the western Himalayas, and the Mediterranean region. This section consists of ~27 herbaceous species with inconspicuous or no staminodial disks and large petals that are either spreading or cup-shaped. Sections Oneapia and Moutan contain only diploid species ($2n = 10$), while one-third of the species in section *Paeonia* are tetraploids (Stern, 1946; Tzanoudakis, 1983; Hong, Zhang, and Zhu, 1988).

Paeonia is a phylogenetically and taxonomically complex group (Stebbins, 1938a; Hong, Zhang, and Zhu, 1988). In particular, section *Paeonia* may have undergone complex reticulate evolution that further obscured phylogenetic relationships (Stebbins, 1948; Sang et al., 1995). Regarding origins of tetraploid species in this section, Barber (1941) and Stern (1946) considered them autotetraploids derived from certain extant diploid ancestors. In contrast, Stebbins (1948) argued that the majority of tetraploid species are allotetraploids, based on observations of bivalents in meiosis of some tetraploid species, such as *P. officinalis*, *P. peregrina*, and *P. wittmanniana*. He further indicated that certain tetraploid species appeared to link gaps of morphological variation among some diploid species, which also suggested hybrid origins of the tetraploids. Later cytogenetic studies confirmed allotetraploid origins of *P. officinalis* and *P. peregrina*, and also revealed *P. parnassica* as an allotetraploid (Tzanoudakis, 1983; Schwarzacher-Robinson, 1986). Recent phylogenetic studies of *Paeonia* using sequences of internal

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[2] Author for correspondence: Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

[3] Current address: Institut für Botanik, Universität Wien, Rennweg 14, A-1030, Vienna, Austria.

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transcribed spacers (ITS) of nuclear ribosomal DNA supported Stebbins' hypothesis and documented reticulate evolution in section *Paeonia* (Sang, Crawford, and Stuessy, 1995). The hybrid species were identified by full or partial ITS sequence additivity of the putative parental species. Partial sequence additivity was suggested to be a result of partial homogenization of parental ITS sequences via gradients of gene conversion (Sang, Crawford, and Stuessy, 1995). Observation of partial homogenization of parental ITS sequence additivity in hybrid species implies the possibility of complete homogenization of parental sequences in certain hybrid species whose hybrid origin, thus, could not be detected by ITS sequences (Wendel, Schnabel, and Seelanan, 1995). Also, diploid hybrid species may have lost one of the parental ITS sequences through segregation. One way to test this hypothesis is to compare the ITS phylogeny with the cpDNA phylogeny of the section. If a hybrid species fixes ITS sequences from one parent via gene conversion, but inherited cpDNA from the other parent, it will have discordant positions on the ITS and cpDNA phylogenies (Rieseberg and Soltis, 1991; Soltis and Kuzoff, 1995; Wolfe and Elisens, 1995).

The coding region of the *mat*K gene, and two intergenic spacers of cpDNA, *trn*L-*trn*F and *psb*A-*trn*H, were sequenced for phylogenetic reconstructions. The *mat*K gene, encoding a maturase, has been suggested as the most rapidly evolving coding region found so far in the chloroplast genome (Neuhaus and Link, 1987; Olmstead and Palmer, 1994). Sequences of the *mat*K coding region have been used to assess phylogenetic relationships at the intrafamilial level in angiosperms, such as within Polemoniaceae (Steele and Vilgalys, 1994) and Saxifragaceae (Johnson and Soltis, 1994). Noncoding regions of cpDNA , which are presumably under less functional constraint and thus evolve more rapidly, may also provide useful phylogenetic information at the lower taxonomical levels (Clegg et al., 1994; Gielly and Taberlet, 1994). A few noncoding regions, including introns and intergenic spacers, have been sequenced recently to assess intrafamilial and intrageneric relationships (Taberlet et al., 1991; Golenberg et al., 1993; Morton and Clegg, 1993; Bohle et el., 1994; Ham et al., 1994; Maner, Natali, and Ehrendorfer,à 1994; Mes and Hart, 1994). The *trn*L–*trn*F intergenic spacer represents the most frequently used noncoding region of cpDNA in phylogenetic studies (Bohle et al., 1994; Gielly and Taberlet, 1994; Ham et al., 1994; Mes and Hart, 1994). The *psb*A–*trn*H intergenic spacer, an evolutionarily plastic region (Aldrich et al., 1988), is employed as a new phylogenetic marker to assess interspecific relationships in *Paeonia*.

Paeonia, with widely disjunct distributions and rich endemism, provides a favorable system for studying historical biogeography of the northern hemisphere. A marked difficulty in understanding historical biogeography of the northern hemisphere is that significant shifts of distributional ranges and extinction of taxa may have been caused by Pleistocene glaciation (Noonan, 1988; Potts and Behrensmeyer, 1992). Pleistocene glaciation was suggested as a primary factor triggering extensive hybridization in section *Paeonia* and subsequently changing its distributions (Stebbins, 1948). Reconstruction of reticulate evolution in the section using ITS sequences reveals that most hybrid species are found in the Mediterranean region, whereas their parental species are restricted to Asia. Detection of parental type of DNA sequences in species of hybrid origin, therefore, provides gene records for historical Mediterranean distributions of the Asian peony species (Sang, Crawford, and Stuessy, 1995).

The primary purposes of this paper, therefore, were to: (1) reconstruct cpDNA phylogeny of *Paeonia*, and assess molecular evolution and phylogenetic utility of coding and noncoding regions of cpDNA; (2) compare the cpDNA phylogeny with the ITS phylogeny of the genus, and particularly of the section *Paeonia* in order to gain new insights into complex reticulate evolution within the section; and (3) discuss biogeography of *Paeonia* based on molecular data and phylogenetic information.

MATERIALS AND METHODS

Thirty-seven accessions of 32 *Paeonia* species were sequenced. For most species, fresh leaves used as sources of DNA were collected from natural populations in Bulgaria, China, Greece, and Spain. The voucher specimens are deposited in OS and UPA. The remaining species were collected from the Royal Botanic Gardens, Kew, and the Beijing Botanical Garden (Table 1). The limited intraspecific sampling is due partly to the remote and endemic distributions, and rarity of most peony species. Particularly in section *Paeonia*, about one-third of species are endemic to a single island, mountain range, or other small area (Stern, 1946; Pan, 1979; Tzanoudakis, 1983). Total DNA was isolated from leaf tissues using the CTAB method (Doyle and Doyle, 1987), and purified in CsCl/ethidium bromide gradients. Double-stranded DNAs were amplified by 30 cycles of symmetric PCR (Sang et al., 1995). The amplification products were purified by electrophoresis through 1.0% agarose gel followed by use of Bioclean (U. S. Biochemical). Purified double-stranded DNAs were used for sequencing reactions employing Sequenase Version 2.0 (Amersham Co., Arlington Heights, IL) (Sang et al., 1995). The sequencing reaction products were separated electrophoretically in 6% acrylamide gel with wedge spacers for 3 h at 1500 V. After fixation, gels were dried and exposed to Kodak XAR x-ray film for 18–48 h. DNA sequences were aligned manually. Sequences of the *mat*K coding region were aligned with those of tobacco and mustard (Neuhaus and Link, 1987).

Primers for amplifying and sequencing the *mat*K coding region and two intergenic spacers are given in Table 2. The forward PCR primer, *mat*K1F, is located ~70 base pairs (bp) upstream of the start codon of the *mat*K coding region and its sequences are conserved between tobacco (Shinozaki et al., 1986) and mustard (Neuhaus and Link, 1987). The reverse PCR primer, *mat*K1R, is similar to the primer, *trn*kR, of Steele and Vilñgalys (1994) except for one more nucleotide at its 5' end. The four internal *mat*K primers are also located in the conserved regions. These six primers have been used successfully to amplify and sequence the *mat*K coding region in *Aesculus* of Hippocastanaceae (except *mat*KR2) (Q. Xiang and D. J. Crawford, unpublished data), *Coreopsis* of Asteraceae (S.-C. Kim and D. J. Crawford, unpublished data), and Hamamelidaceae (except matKF3) (J. Li, unpublished data, University of New Hampshire, Durham, NH).

The forward primers (psbAF) for amplifying the *psb*A–*trn*H intergenic spacer were designed in a region of the *psb*A genes conserved between tobacco of Solanaceae (Shinozaki et al., 1986) and *Brassica napus* of Brassicaceae (Genbank No. M36720). The reverse primer (*trn*HR) is in a region of the *trn*H gene that is conserved among tobacco, *Helianthus annuus* of Asteraceae (Genbank No. X60428), and *Arabidopsis thaliana* of Brassicaceae (Genbank No. X79898). The primers for the *trn*L–*trn*F intergenic spacer are similar to those of Taberlet et al. (1991). The reverse primer, trnFR, has one more nucleotide at the

TABLE 1. Accessions studied for DNA sequences. Classification of *Paeonia* is based on Stern (1946) and later alterations (Fang, 1958; Pan, 1979; Tzanoudakis, 1983; Pei, 1993). *, tetraploid; #, both diploid and tetraploid populations known; ?, ploidy level unknown (Stern, 1946; Tzanoudakis, 1983; Hong, Zhang, and Zhu, 1988). Leaf samples were obtained from field, the Royal Botanic Gardens, Kew, or Beijing Botanic Garden (BBG). For taxa obtained from botanical gardens, localities indicate source of introduction.

Taxon	Collection number	Locality	Abbreviations
Section *Moutan* DC.			
Subsection *Vaginatae* Stern			
P. rockii (Haw et Lauener) Hong et Li	Sang 104	Mt. Taibei, Shaanxi Prov., China	ROC
P. suffruticosa ssp. *spontanea* Rehd.	Pei 9201001	Mt. Ji, Shaanxi Prov., China	SPO
P. szechuanica Fang	Sang 225	Marekang Co., Sichuna Prov., China	SZE
Subsection *Delavayanae* Stern			
P. delavayi Franch.	Sang 186	Lijiang Co., Yunnan Prov., China	DEL
P. lutea Delavay ex Franch.	Sang 125	Mt. Xi, Yunnan Prov., China	LUT1
	Sang 272	Bomi Co., Tibet, China	LUT2
Section *Onaepia* Lindley			
P. brownii Dougl. ex Hook.	Bartholomew 6708	Modoc Co., California, USA	BRW
P. californica Nutt. ex Torr. et Gray	Sang 583	Los Angeles Co., California, USA	CAL
Section *Paeonia*			
Subsection *Foliolatae* Stern			
**P. arietina* Andr.	Kew 1968-19121	Turkey	ARI
**P. banatica* Rochel	Kew 1947-48101	Banati, Bazsarozsa, Hungary	BAN
P. broteri Boiss. et Reut.	Sang 704	Sierra Nevada, Granada, Spain	BRT
P. cambessedesii Willk.	Kew 69-17456	Balearic Islands, Spain	CAM
**P. coriacea* Boiss.	Sang 701	Sierra Nevada, Granada, Spain	COR
P. emodi Wall. ex Royle	Kew 1966-7902	India	EMO
P. japonica (Makino) Miyabe & Takeda	BBG	Japan	JAP
P. lactiflora Pallas	Hong 85006	Chicheng Co., Hebei Prov., China	LAC
#*P. mairei* Levelle	Sang 351	Mt. Taibei, Shaanxi Prov., China	MAI
**P. mascula* ssp. *hellenica* Tzanoud.	Tzanoudakis	Holkis, Greece	MASH
ssp. *mascula* (mill.) Tzanoud.	Tzanoudakis	Greece	MASM
P. mlokosewitschi Lomak.	Kew 579-56.57915	Caucasus	MLO
#*P. obovata* Maxim.	Sang 352	Mt. Taibei, Shaanxi Prov., China	OBO
**P. parnassica* Tzanoud.	Sang 684	Mt. Panassos, Greece	PAR
P. rhodia Stern	Sang 688	Island Rhodes, Greece	RHO
**P. russi* Bivona	Kew 1974-4075	Islands of western Mediterranean	RUS
?*P. sterniana* Fletche	Kew 1962-37101	Southeastern Tibet	STE
**P. wittmanniana* Hartwiss ex Lind.	Kew 69-18448	Caucasus	WIT
Subsection *Paeonia*			
P. anomala L.	Sang 414	Yiling Co., Xinjiang Prov., China	ANO
#*P. clusii* Stern	Sang 661	Island Crete, Greece	CLU
?*P. humilis* Retzius	Aallali	Seno de Los Carceles, Granada, Spain	HUM1
	Kew 1969-18516	Southern France	HUM2
**P. officinalis* L.	Kew 481-617-48101	Europe	OFF1
	Rowland	Central Croatia	OFF2
**P. peregrina* Miller	Sang 636	Sofia, Bulgaria	PER1
	Sang 642	Lefkas, Greece	PER2
P. tenuifolia L.	Sang 610	Sofia, Bulgaria	TEN
P. veitchii Lynch	Sang 101	Mt. Taibei, Shaanxi Prov., China	VEI1
	BBG	Mongda Co., Qinghai Prov., China	VEI2
P. xinjiangensis Pan	Sang 462	Aletai Co., Xinjiang Prov., China	XIN

TABLE 2. Primers designed to amplify and sequence *mat*K coding region and two intergeneric spacers in *Paeonia*. Positions of primers correspond to nucleotide positions of cpDNA of tobacco (Shinozaki et al., 1986).

Primers		5′ Sequences 3′	Position
*mat*K			
Forward	matK1F	ACTGTATCGCACTATGTATCA	B 3727–3707
	matK2F	GTTCACTAATTGTGAAACGT	B 3495–3476
	matK3F	AAGATGCCTCTTCTTTGCAT	B 3141–3122
Reverse	matK1R	GAACTAGTCGGATGGAGTAG	B 1834–1853
	matK2R	TTCATGATTGGCCAGATCA	B 2132–2150
	matK3R	GATCCGCTGTGATAATGAGA	B 2391–2410
*psb*A–*trn*H			
Forward	psbAF	GTTATGCATGAACGTAATGCTC	B 608–587
Reverse	trnHR	CGCGCATGGTGGATTCACAAATC	B 28–49
*trn*L–*trn*F			
Forward	trnLF	AAAATCGTGAGGGTTCAAGTC	A 49853–49873
Reverse	trnFR	GATTTGAACTGGTGACACGAG	A 50292–50272

5' end of the primer f of Taberlet et al. (1991). The forward primer (trnLF) is designed ten nucleotides farther away from the 3' end of the *trn*L 3' exon than the primer e of Taberlet et al. (1991) in order to read sequences closer to the 5' end of the intergenic spacer.

Sequence divergence between species for each cpDNA region was calculated using DNADIST program of PHYLIP version 3.5c (Felsenstein, 1994). The Jukes-Cantor model was used for correcting possible multiple hits of nucleotide substitutions (Jukes and Cantor, 1969).

Phylogenetic analyses were performed initially using the *mat*K coding region and the *psb*A–*trn*H intergenic spacer independently, and then using the combined data set from mutations in the three cpDNA regions. Mutations, including nucleotide substitutions and insertions/deletions (indels), were analyzed by unweighted Wagner parsimony using PAUP version 3.1.1 (Swofford, 1993). Indels were coded as binary characters in the analysis. The shortest trees were searched with TBR Branch Swapping of the heuristic method, and character changes were interpreted with the ACCTRAN optimization. Bootstrap analyses were carried out with 500 replications using TBR Branch Swapping of the Heuristic search (Felsenstein, 1985). Section *Oneapia* of *Paeonia* was chosen as the outgroup for the cladistic analysis of the genus (Watrous and Wheeler, 1981; Maddison, Donoghue, and Maddison, 1984; Sang, Crawford, and Stuessy, 1995).

Discordance between the ITS and *mat*K phylogenies was assessed by two methods, inspection and assessment of support, and the Templeton test (Mason-Gamer and Kellogg, in press). These two methods are chosen because they can assess conflicts involving individual clades between the trees. The method of inspection and assessment of support compares bootstrap support for a certain clade in two data sets. For example, when a clade is strongly supported by a bootstrap value of 90% on tree A, but does not appear on tree B, we can check the table of ''partitions found in one or more trees and frequency of occurrence'' from the PAUP 3.1.1 bootstrap output of tree B for any possible support for this clade. The lower the support, the higher is the conflict for formation of this clade between the two data sets (Mason-Gamer and Kellogg, in press). The Templeton test forces data set A to generate the tree topology obtained from data set B, and tests whether the resulting tree is significantly less parsimonious than the most parsimonious tree obtained from data set A under no constraint (Templeton, 1983; Larson, 1994). When tree B contains only one resolved clade that is used as the constraint topology for parsimony analysis of data set A, the significant level of support of this clade by data set A is tested (Mason-Gamer and Kellogg, in press). Because only ITS sequences that did not show additivity were used in parsimony analysis (Sang, Crawford, and Stuessy, 1995), the *mat*K data set is reduced to match the same species in the ITS data set for comparison.

RESULTS

The entire *mat*K coding region is 1491 bp long, and nucleotide substitutions were found at 53 nucleotide sites among all the peony species. No nucleotide substitutions were found between sequences of different accessions of the same species. Parsimony analyses generated five equally most parsimonious trees with length of 59, a consistency index (CI) of 0.949, and a retention index (RI) of 0.972. A strict consensus tree of these five trees was computed and has a tree length of 62, a CI of 0.903 (0.842 excluding autapomorphies), and a RI of 0.945 (Fig. 1).

Aligned sequences of the *psb*A–*trn*H intergenic spacer of *Paeonia* species are shown in Fig. 2. The sequences of two populations of *P. lutea* differ by one nucleotide substitution. For the remaining species, different accessions of each species have identical sequences. With this straightforward sequence alignment, the length of the intergenic spacer varies from 281 bp (*P. mairei*) to 324 bp (*P. spontanea* and *P. szechanica*). A total of 31 variable sites (with nucleotide substitutions) and 13 indels occur among these species. Twenty-four variable sites and 11 indels were used for reconstructing phylogeny and nine equally most parsimonious trees, with a length of 36, CI of 0.946, and RI of 0.981, were obtained (see Discussion for reasons for excluding some mutations from the phylogenetic analysis). A strict consensus tree with a length of 37, CI of 0.921 (0.875 excluding autapomorphies), and RI of 0.971, was generated (Fig. 3).

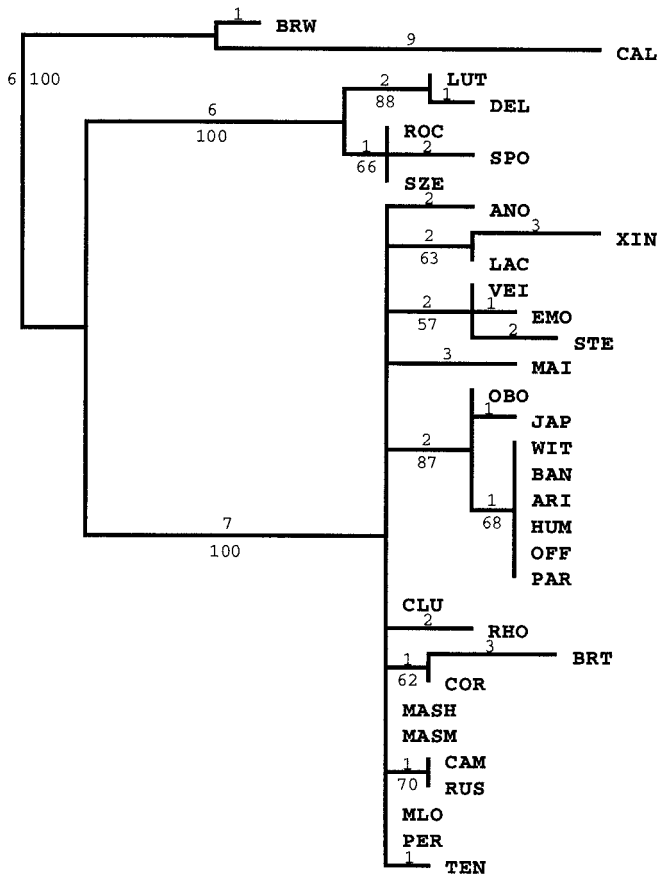

Fig. 1. Strict consensus tree of five equally most parsimonious trees of *Paeonia* generated from sequences of *mat*K coding region of chloroplast DNA. Tree length = 62, CI = 0.903 (0.842 excluding autapomorphies), RI = 0.945. Numbers above branches represent nucleotide substitutions; numbers below branches represent bootstrap values. For species abbreviations, see Table 1.

For the *trn*L–*trn*F intergenic spacer, because the forward primer is still very close to the 3' end of the *trn*L 3' exon, about ten nucleotides at the 5' end of the spacer could not be read. Sequences of different accessions of the same species are identical. Length of the aligned sequences varies from 372 bp (*P. obovata*) to 404 bp (*P. sterniana*). Among all species, only nine variable sites and five indels were detected (Table 3). Phylogenetic reconstruction was not performed for this region alone because of little phylogenetic information.

Phylogenetic analysis of combined mutations from the *mat*K coding region and the two intergenic spacers generated 102 equally most parsimonious trees. The consen-

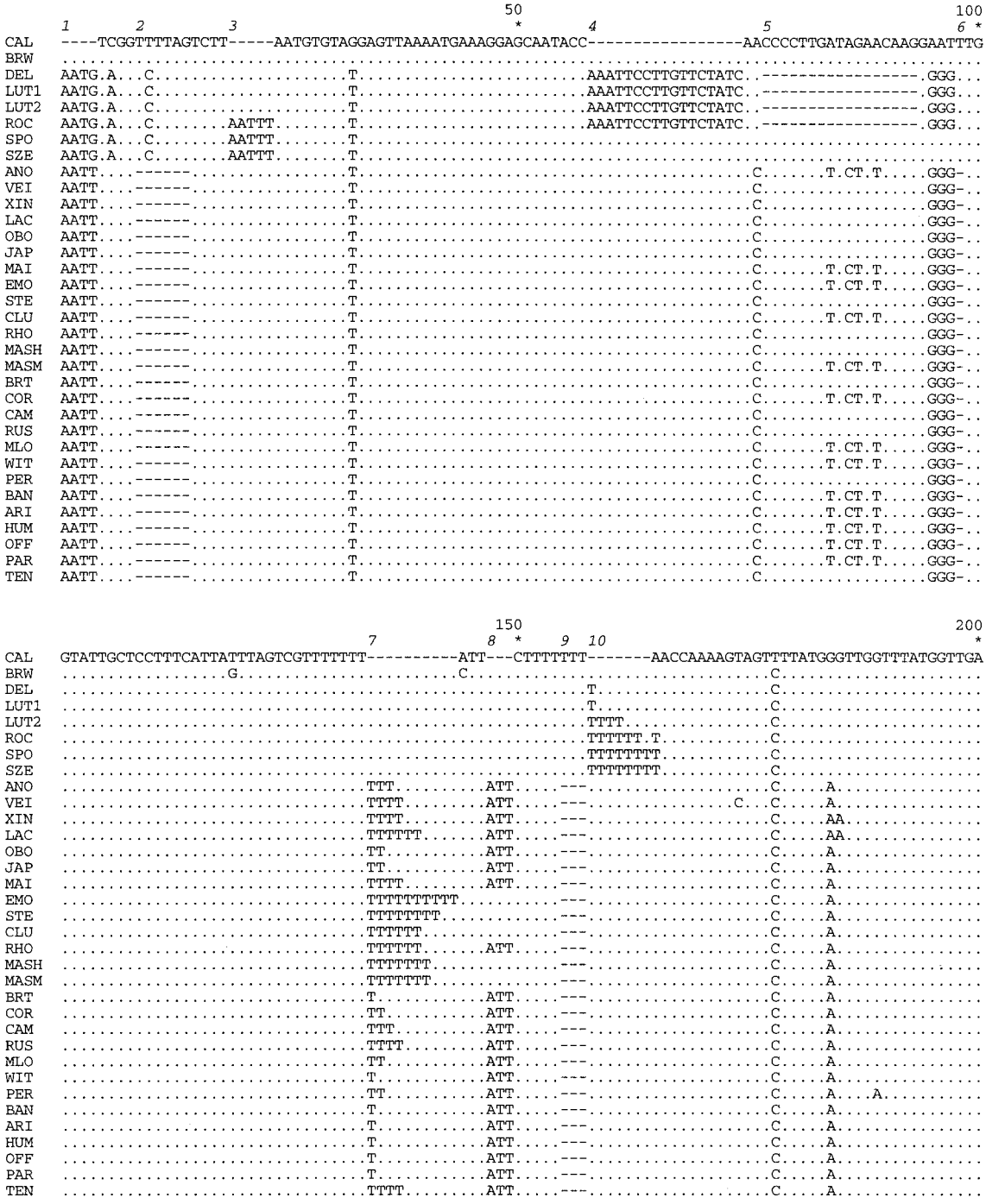

Fig. 2. Aligned sequences of *psb*A–*trn*H intergenic spacer of chloroplast DNA in *Paeonia*. Dots indicate the same nucleotide as in *P. californica*; dashes indicate gaps. Asterisks and numbers indicate positions of nucleotides that are numbered consecutively from the 5′ to 3′. Italic numbers indicate insertions/deletions numbered consecutively from 5′ to 3′.

sus tree, with a CI of 0.855 (0.769 excluding autapomorphies) and a RI of 0.926, differs topologically from the *mat*K phylogeny by only two clades in section *Paeonia* (tree not shown). The clade containing *P. veitchii, P. emodi,* and *P. xinjiangensis* on the *mat*K phylogeny collapsed on the combined tree, and a new clade containing *P. clusii* and *P. mascula* formed on the combined tree.

Comparisons of sequence divergence and phylogenetic information from variable sites among the two intergenic spacers and the *mat*K coding region are given in Table 4. Comparisons of phylogenetic information from indels and relative frequency of indels vs. nucleotide substitutions between the two intergenic spacers are given in Table 5. Average percentage sequence divergences of *mat*K, the *psb*A–*trn*H intergenic spacer, and ITS were compared within and among sections of *Paeonia* (Table 6). Com-

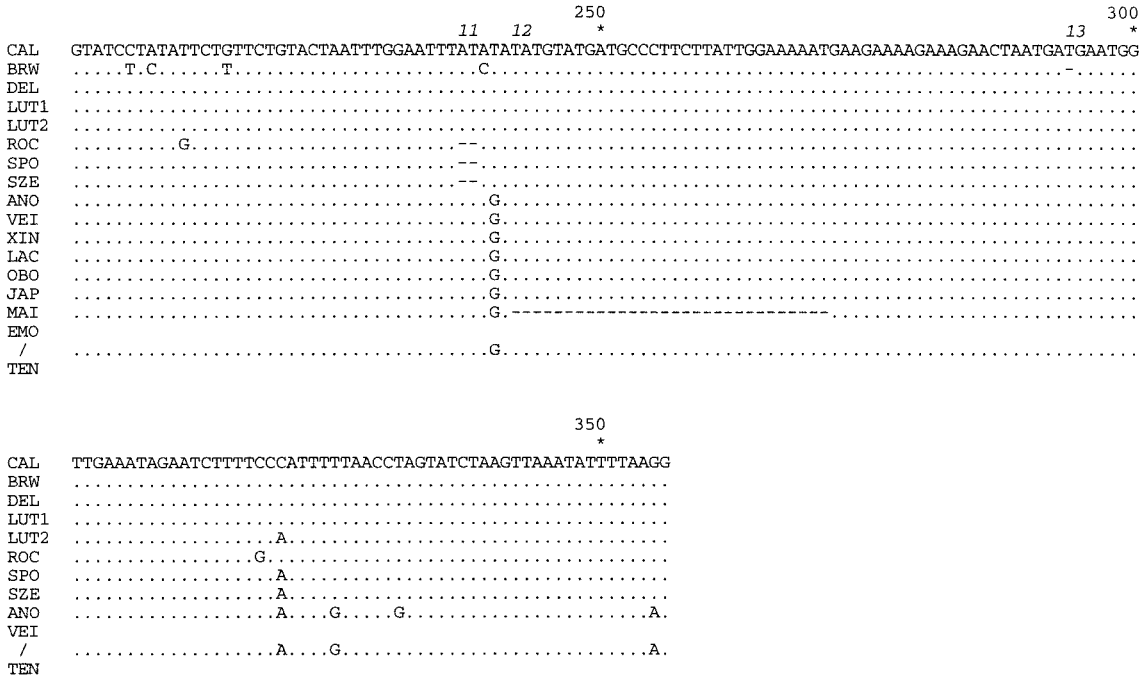

Fig. 2. Continued.

parisons of sequence divergences indicate that ITS sequences evolve slightly more rapidly than the *psb*A–*trn*H intergenic spacer, and over three times more rapidly than the *mat*K coding region.

Comparisons of the phylogenetic tree generated from the ITS data set and the reduced *mat*K data set are illustrated in Fig. 4. The bootstrap values from the two data sets were compared for all the clades, and suggest highly conflicting support for several clades of section *Paeonia* on either tree (Fig. 4I, II). The two well-supported basal clades of section *Paeonia* on the ITS tree received no support from the *mat*K sequences. The strongly supported sister group relationship of *P. veitchii* and *P. xinjiangensis* on the ITS tree receives no support from the *mat*K data. The monophyly of *P. tenufolia* together with *P. arietina, P. humilis, P. officinalis,* and *P. parnassica* was supported by 100% bootstrap on the ITS tree, but 0% by the *mat*K data. On the *mat*K phylogeny, a well-supported monophyletic group (*P. obovata, P. japonica, P. arietina, P. humilis, P. officinalis,* and *P. parnassica*) had only 0.3% bootstrap support from the ITS data set. A group of four species (*P. arietina, P. himilis, P. officinalis,* and *P. parnassica*), which have identical ITS and *mat*K sequences, was supported by 65% bootstrap value on the *mat*K tree, but on the ITS phylogeny they never formed a monophyletic group by themselves without *P. tenuifolia.* Likewise, the moderately supported (63%) sister group relationship between *P. lactiflora* and *P. xinjiangensis* on the *mat*K tree was not supported at all by the ITS data without the involvement of *P. veitchii.*

For the Templeton test, two basal clades of section *Paeonia* and a clade consisting of *P. lactiflora* and *P. xinjiangensis,* which are found only on the ITS tree, were used as constraint topology for generating parsimonious trees from the reduced *mat*K data set (Fig. 4I). Similarly, each of three clades found only on the *mat*K tree was used as constraint topology for the ITS data set (Fig. 4II). In none of the cases, however, was the exact constraint topology obtained from parsimony analysis (Fig. 4A—E). Examination of the characters of each data set indicates that not a single character in a data set can serve as a synapomorphy for a clade obtained only from the other data set. This result supports the comparative bootstrap values in demonstrating that the two data sets highly conflict in supporting each of these clades. In the first test (Fig. 4A), it is significantly less parsimonious ($P < 0.005$, two-tailed value) to form a clade including *P. japonica, P. obovata, P. humilis, P. officinalis, P. arietina,* and *P. tenuifolia* with the ITS data set, although the cost of taking out *P. tenuifolia* is still impossible to measure. The results of the remaining four tests, however, are not significant because the number of characters undergoing step changes during each constraint analysis is smaller than the minimal requirement of five characters in order to get significant test results (Fig. 4B-E). Such results may be due to the failure of obtaining the constraint topology after parsimony analysis. In each of the cases, only the cost of breaking down a certain clade is taken into account while formation of a desired clade was not achieved, and thus the cost could not be estimated.

DISCUSSION

Inversions in the psbA–trnH intergenic spacer—The simple alignment of sequences between nucleotide sites 57 and 97 of the *psb*A–*trn*H intergenic spacer (Fig. 2) may not be an appropriate evolutionary interpretation. This region contains a pair of 20 or 27 bp exact inverted repeats, which are separated by 21 or 6 bases, respectively (Fig. 5A). Three types of sequences can be recognized: type I occurs in section *Oneapia,* and *P. suffruticosa* ssp. *spontanea* and *P. szechuanica* of subsection

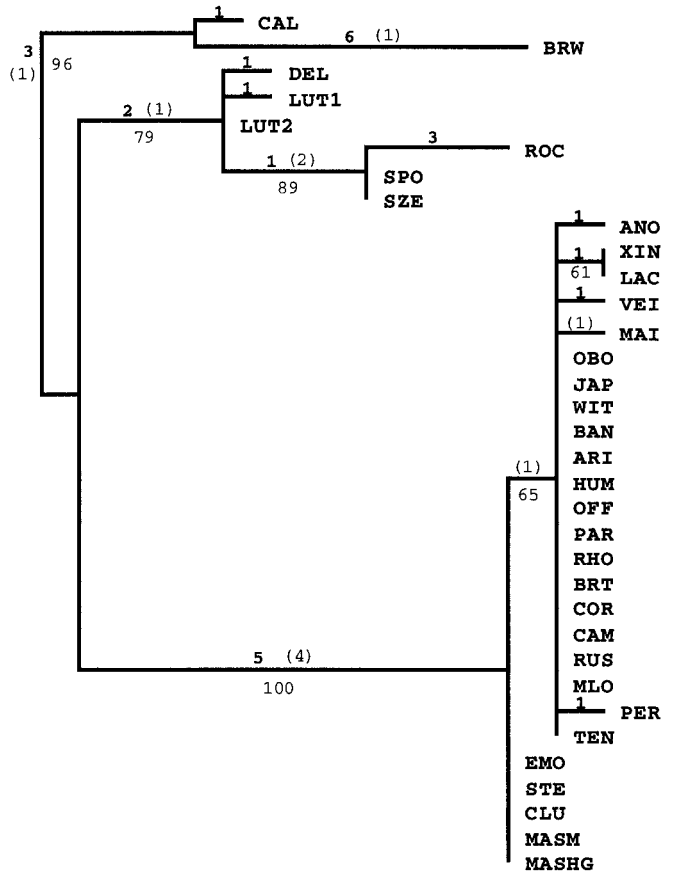

Fig. 3. Strict consensus tree of nine equally most parsimonious trees obtained from sequences of *psb*A–*trn*H intergenic spacer of chloroplast DNA. Tree length = 38, CI = 0.921 (0.875 excluding autapomorphies), RI = 0.971. Above branches, bold numbers represent nucleotide substitutions, numbers in parentheses represent number of indels; numbers below branches represent bootstrap values.

TABLE 3. Mutations in *trn*L–*trn*F intergenic spacer.

Taxa[a]	Mutation	Sequence	Position[b]
Sect. *Paeonia*	Insertion	TTTT	39–42
STE	Insertion	TT	43–44
OBO	Deletion	32 bp[c]	61–92
Sect. *Oneapia*	Insertion	TATACC	220–225
MAI	Deletion	26 bp[d]	270–295
Sect. *Oneapia*	Insertion	TT	274–275
OBO, STE, WIT	Transversion	G–T	45
BAN, ARI, HUM			
OFF, PAR			
COR	Transversion	A–C	120
Sect. *Paeonia*	Transition	A–G	137
CLU, MASH, MASM	Transversion	T–G	154
CAL, ROC	Transition	C–T	192
XIN, LAC	Transition	T–C	239
CAL	Transition	T–C	266
ROC, SPO, SZE	Transition	G–A	297
CAL	Transition	C–T	315
PER	Transition	G–A	395

[a] Species names are abbreviated as in Table 1.
[b] Nucleotide sites in aligned sequences are numbered consecutively from 5′ to 3′.
[c] ATTCATTATGTTTATCATTTATTCTACTCTTT.
[d] TTTTTTGAAGATCCAAGAAATTCCAG.

Vaginatae; type II occurs in subsection *Delavayanae* and *P. rockii*; and type III is found in section *Paeonia*, which further includes two subtypes i and ii (Fig. 5A). Type I and II sequences can be converted into eachother by inversions of sequences bordered by the inverted repeats. Similarly, in type III sequence, an inversion of the sequence bordered by the inverted repeats in one subtype sequence can give rise to the other subtype. The inversions occur quite frequently and homoplasiously within the genus (Fig. 2). In contrast to some large inversions in cpDNA that provided reliable phylogenetic information at the higher taxonomic levels (Jansen and Palmer, 1987; Doyle et al., 1992; Rauberson and Jansen, 1992), short inversions in the intergenic spacer easily yield homoplasious information even at the interspecific level and thus should not be included in phylogenetic analyses.

The mechanism responsible for change between the type III sequence and the other two types is more complex. In the type I sequence, there is another pair of short inverted repeats in the region between the long inverted repeats (Fig. 5B). Therefore, a stem-loop structure with two stems and two loops can be formed (Fig. 5B). The evolutionary changes that are likely to have occurred in the small loop between the two stems include deletion of the T and two transitions of A to G to match the two Cs so that a single longer stem of the type III sequence could be formed. The two substitutions in this small loop are facilitated by this particular stem-loop structure, and thus

TABLE 4. Comparisons of sequence divergence and phylogenetic information from variable sites among two intergenic spacers and *mat*K coding region.

CpDNA regions	Percentage sequence divergence[a]	Number of variable sites	Number of informative sites[b]	Percentage informative sites[c]	Number of homoplasious sites[d]	Number of synapomorphic sites[e]	Percent synapomorphic sites[f]
*psb*A–*trn*H	1.29	24	13	54.2	1	12	92.3
*trn*L–*trn*F	0.39	10	6	60.0	2	4	66.7
*mat*K	0.58	53	30	56.6	3	27	90.0

[a] Average of percentage pairwise sequence divergences estimated using the Jukes-Cantor model. The same species were sequenced for these three regions.
[b] At a phylogenetically informative site, a nucleotide substitution is shared by two or more species.
[c] Percentage of phylogenetically informative sites among the total number of variable sites.
[d] Homoplasious sites of a region are those where nucleotide substitutions phylogenetically conflict with other substitutions in this region, and also the ones that conflict with phylogenies obtained from other DNA regions (e.g., site 45 of *trn*L–*trn*F intergenic spacer comparing with *mat*K and ITS phylogenies).
[e] Difference between number of informative sites and number of homoplasious sites.
[f] Percentage of synapomorphic sites among informative sites.

TABLE 5. Comparisons of phylogenetic information of indels and number of indels vs. number of variable sites in two intergenic spacers.[a]

Intergenic spacers	Number of indels	Number of informative indels	Percentage informative indels	Number of homoplasious indels[b]	Number of synapomorphic indels	Ratio of indels to variable sites[c]	Ratio of syn. indels to syn. sites[d]
*psb*A–*trn*H	11	9	81.8	1	8	0.46	0.67
*trn*L–*trn*F	6	3	50.0	0	3	0.60	0.75

[a] There is no indel in *mat*K coding region of *Paeonia*. For definitions of columns see also Table 4.
[b] Indel 8 of *psb*A–*trn*H intergenic spacer conflicts with *mat*K and ITS phylogenies.
[c] Ratio of number of indels to number of variable sites found in each intergenic spacer (Table 4).
[d] Ratio of number of synapomorphic indels to number of synapomorphic sites in each intergenic spacer (Table 4).

should not be treated as regular substitutions in calculating sequence divergence in order to avoid overestimating rates of sequence divergence. Therefore, they were not taken into account in calculating sequence divergence or reconstructing phylogeny. Likewise, the two substitutions, A to C at site 76 and T to G at site 97, are at the corresponding positions of the inverted repeats, and treated as only one substitution for sequence divergence estimation and phylogenetic reconstruction.

Insertions/deletions in the intergenic spacers—Of 11 indels in the *psb*A–*trn*H intergenic spacer, four are perfect (indels 8 and 11) or imperfect (indels 2 and 3) duplications or deletions of prior duplications of adjacent sequences (Fig. 2). Slipped-strand mispairing is most likely the mechanism responsible for this type of indel (Levinson and Gutman, 1987). Indels 7, 9, and 10, which are duplications or deletions of a portion of poly(T) tracks, may also result from slipped-strand mispairing (Wolfson, Higgins, and Sears, 1991). Since the probability of occurrence of further insertions or deletions increases as the track of repetitive nucleotide sequences gets longer (Streisinger and Owen, 1985; Golenberg et al., 1993), multiple indels must have occurred at indels 7 and 9 to create the pattern of differential lengths of poly(T) tracks. In the *trn*L–*trn*F intergenic spacer, three of six indels are also portions of poly(T) tracks.

Apparently, indels in these two intergenic spacers occur less frequently and provide a smaller amount of phylogenetic information than nucleotide substitutions (Table 5). As phylogenetic characters, indels do not conflict with each other or with nucleotide substitutions in either of the two intergenic spacers. Only indel 8 of the *psb*A–*trn*H intergenic spacer conflicts with relationships in the *mat*K phylogenies (Figs. 1, 2). Overall, the indels in the cpDNA intergenic spacers are quite reliable phylogenetic characters in *Paeonia*, which is concordant with findings in other plant groups at the lower taxonomical levels (Ham et al., 1994; Mes and Hart, 1994). However, indels may not be reliable phylogenetic characters at higher taxonomic levels because the chance of superimposition of indels increases as divergence time increases (Morton and Clegg, 1993; Golenberg et al., 1993).

Nucleotide substitutions in cpDNA coding and noncoding regions—A comparison of average species pairwise sequence divergence in the two intergenic spacers and the *mat*K coding region (Table 4) indicates that the *psb*A–*trn*H intergenic spacer has much higher rates of nucleotide substitutions than the other two regions. The *trn*L–*trn*F intergenic spacer has lower substitution rates than the *mat*K coding region, suggesting that higher substitution rates should not always be expected in noncoding regions than in coding regions of cpDNA. Distinguishing autapomorphic, synapomorphic, and homoplasious substitutions in the intergenic spacers and the *mat*K coding region should enable comparisons of the quality of phylogenetic information yielded from these regions (Table 4). The percentage of phylogenetically informative sites (the sites where substitutions are shared by two or more taxa) among the variable sites is similar for the three regions. The percentage of synapomorphic sites among informative sites is highest in the *psb*A–*trn*H intergenic spacer (92.3%), and lowest in the *trn*L–*trn*F intergenic spacer (66.7%). Therefore, the *psb*A–*trn*H spacer, which evolves most rapidly among the three regions and provides best synapomorphic information, should be a useful region for phylogenetic studies at the lower taxonomical levels. The *mat*K coding region, although evolving about twice as slowly as the *psb*A–*trn*H spacer, has over twice as many synapomorphic sites as the intergenic spacer because it is about four times longer. In comparison with the ITS phylogeny, the *mat*K coding region provides a comparable amount of information for phylogenetic reconstruction, e.g., a similar number of substitutions is found to support each section of the genus (Figs. 1, 6), and may serve as a good marker for phylogenetic studies at the intrageneric level. The most frequently used intergenic spacer, *trn*L–*trn*F, however, evolves most slowly and homoplasiously, and thus its phylogenetic utility at the intrageneric level is questionable.

Phylogenetic reconstruction—Phylogenies reconstructed from sequences of the *mat*K coding region and *psb*A–*trn*H intergenic spacer are resolved and congruent at the sectional level (Figs. 1, 3). Two subsections of

TABLE 6. Average percentage sequence divergence of ITS, *mat*K, and *psb*A–*trn*H intergenic spacer within and among sections of *Paeonia*. Letters *M, O,* and *P* represent sections *Moutan, Oneapia,* and *Paeonia,* respectively.

Sequence	Within *Oneapia*	Within *Moutan*	Within *Paeonia*	Between *O* and *M*	Between *O* and *P*	Between *M* and *P*	Between *O* and *M&P*	Between *M* and *O&P*	Between *P* and *O&M*
ITS	2.94	0.83	2.10	4.03	4.62	3.64	4.38	3.69	3.89
*psb*A–*trn*H	2.30	0.59	0.64	2.59	3.67	2.87	3.47	2.85	3.07
*mat*K	0.74	0.25	0.27	1.18	1.35	1.11	1.33	1.12	1.18

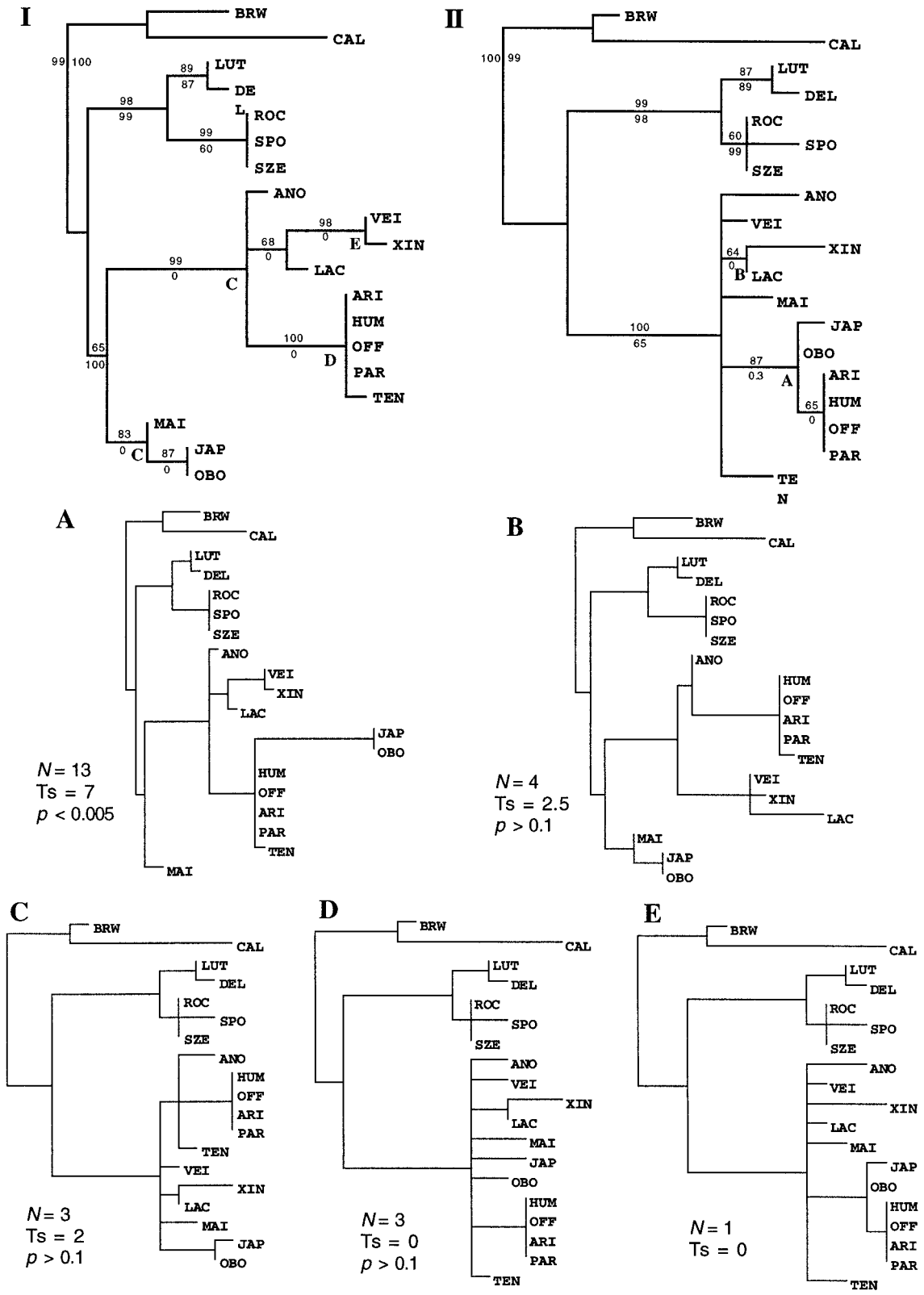

Fig. 4. Comparison of discordance between ITS and *mat*K phylogenies using methods of inspection and assessment of support (I and II) and Templeton test (A - E). I and II, phylogenetic trees generated from ITS sequences (Sang, 1995) and *mat*K sequences, respectively. Numbers above branches represent bootstrap support (1000 replications) obtained from the data set generating this tree; numbers below branches represent bootstrap support (1000 replications) obtained from the other data set. Letters A - E indicate the clades that are used as constraint topology in Templeton test. A -E, consensus trees of the most parsimonious trees generated under constraint of clades A - E (I and II), respectively. *N*, number of hcaracters undergoing step changes after constraint analysis; Ts, test statistic; *p*, probability.

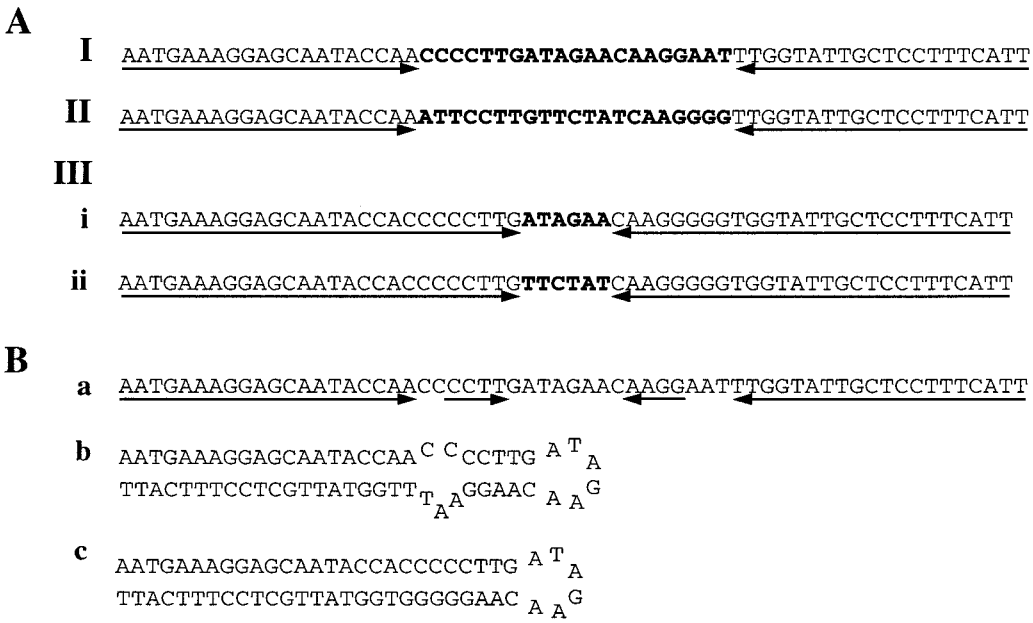

Fig. 5. (A) Three types of sequences of region between nucleotide sites 40 and 117 of *psb*A–*trn*H intergenic spacer (Fig. 2). Lines and arrows below seuqences indicate inverted repeats. Nucleotides (boldface letters) that are bordered by inverted repeats have undergone inversions. (B) (a) Type I sequence containing another short inverted repeats between the long inverted repeats; (b) stem-loop structure of type I sequence; (c) stem-loop structure of type III (i) sequence.

section *Moutan* are also recognized in the cpDNA phylogenies. Lower resolution of relationships within section *Paeonia* is probably due to the relatively recent origins of the species and reticulate evolution that led to the loss of one divergent cpDNA (see later discussion). The CI (0.903) and RI (0.945) of the *mat*K consensus tree are high, suggesting that the phylogenetic reconstruction is fairly reliable. In fact, only three characters (variable sites) out of 53 characters are homoplasious for the parsimony analysis. The phylogenetic analysis of the *psb*A–*trn*H intergenic spacer resolved only two clades within section *Paeonia*. The clade containing 22 species supported only by indel 8 conflicts with relationships on the *mat*K phylogeny (Figs. 1, 2). It is very unlikely that this clade reflects the true cpDNA phylogeny, but is just a result of random deletions of the ATT duplication in *P. emodi, P. sterniana, P. clussi,* and two subspecies of *P. mascula.* This homoplasious mutation is also responsible for the collapse of the *mat*K clade containing *P. veitchii, P. emodi,* and *P. sterniana* on the tree generated from the combined data set.

The *trn*L–*trn*F intergenic spacer provides very limited phylogenetic information (Table 3). Two indels distinguish section *Oneapia* from the other two sections. One indel and one nucleotide substitution serve as synapomorphies for section *Paeonia.* One substitution defines subsection *Vaginatae.* One substitution supports the sister relationship of *P. lactiflora* and *P. xinjiangensis,* as on the *psb*A–*trn*H spacer and *mat*K phylogenies. One shared substitution by *P. californica* and *P. rockii,* however, is clearly homoplasious. Another apparent homoplasious substitution shared by eight species (OBO, STE, WIT, BAN, ARI, HUM, OFF, and PAR) may result from a synapomorphic substitution that defines the monophyletic group on the *mat*K phylogeny (JAP, OBO, WIT, BAN, ARI, HUM, OFF, and PAR) followed by a reversal substitution in *P. japonica*. This explanation is in agreement with the *mat*K and ITS phylogenies where *P. japonica* is a sister group of *P. obovata.* The reason why *P. sterniana* has this substitution is unclear.

Overall, the *mat*K coding region served as a better phylogenetic marker for resolving close specific relationships in *Paeonia* than the intergenic spacers. The *mat*K phylogeny will be compared with the ITS phylogeny for a better understanding of the species phylogeny of the genus.

DNA sequence and morphological divergence—Evolutionary tempos may or may not be concordant at molecular and morphological levels (Sytsma and Smith, 1992). Among three sections of *Paeonia,* section *Oneapia* is the most distinct one at the molecular level. A comparison of sequence divergence between any one section and the other two indicates that section *Oneapia* has the highest percentage sequence divergence values of ITS (4.38), *psb*A–*trn*H intergenic spacer (3.74), and *mat*K (1.33) (Table 6). The earliest evolutionary split within the genus *Paeonia* might have occurred between section *Oneapia* and the other two sections if the molecular clock is assumed. Morphologically, section *Oneapia* is also distinct from the other two sections by its small flowers (2–3 cm in diameter vs. $>$ 5 cm in sections *Moutan* and *Paeonia*) with fleshy and strongly concave petals.

Within section *Oneapia,* however, rates of divergence between DNA sequences and morphology are strikingly different. Sequence divergence is higher within section *Oneapia* than within any other two sections (Table 6). The two species of section *Oneapia* are distributed allopatrically, i.e., *P. californica* is endemic to southern California, and *P. brownii* is found from northern California

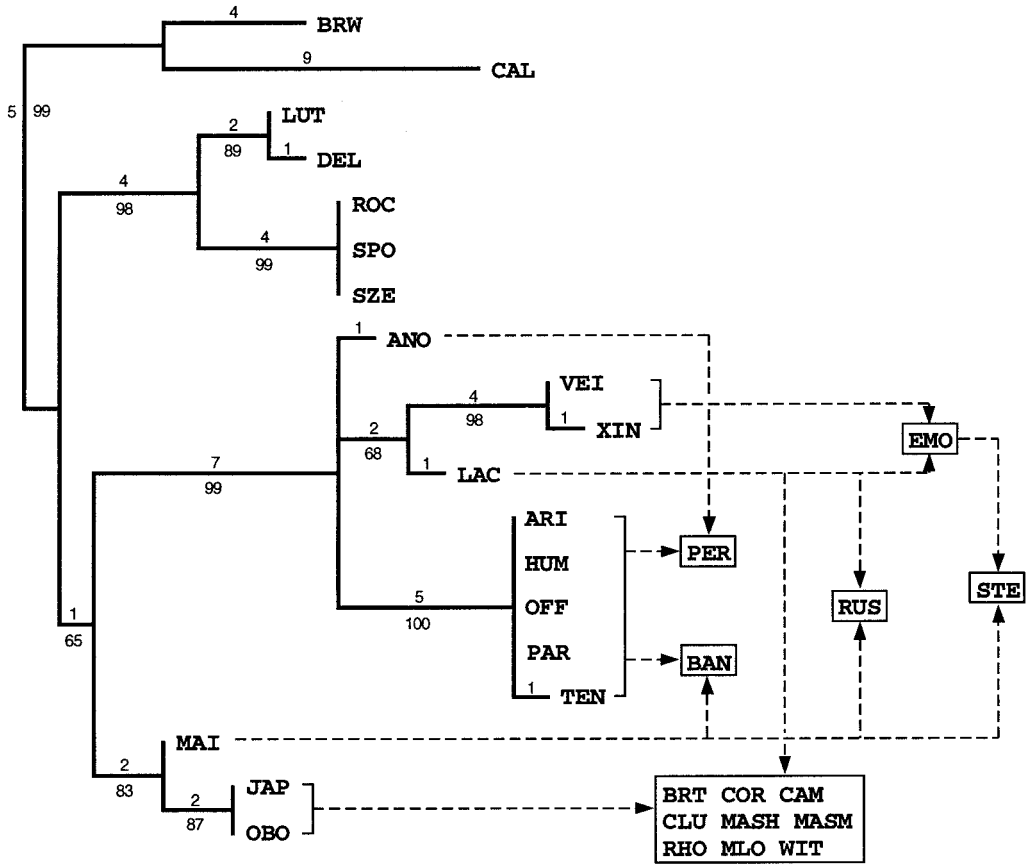

Fig. 6. Phylogeny of *Paeonia* generated from ITS seuqences (Sang, 1995; Sang, Crawford, and stuessy, 1995). Solid lines in the left portion of the figure represent relationships of species that do not have additive ITS sequences, which is a strict consensus tree of two equally most parsimonious trees and has a consistency index of 0.911 and a retention index of 0.959. Numbers above lines indicate nucleotide substitutions; numbers below branches represent bootstrap values. The species boxed in the right portion of the figure have nucleotide additivity at the sites that are variable between other species, and they are considered to be of hybrid origin. The dashed lines lead to the putative parents of the hybrid species based on sequence additivity.

to British Columbia. *Paeonia* californica is adapted to warmer and wetter climates and flowers from February to April, whereas *P. brownii* is semixerophytic and flowers during June and July (Stebbins, 1938b). However, these two species are morphologically very similar to each other and had been treated as one species until detailed morphological, ecological, and cytological studies that suggested they have undergone considerable genetic divergence rather than only morphological modification due to ecological factors (Stebbins, 1938b; Stebbins and Ellerton, 1939). DNA sequence data support this hypothesis and indicate that morphological evolution in section *Oneapia* has been remarkably slow compared with the high level of sequence divergence.

Within subsection *Vaginatae* of section *Moutan*, however, morphological divergence apparently exceeds DNA sequence divergence. Three species studied by DNA sequences are morphologically distinct and allopatrically distributed, but have identical ITS sequences. In addition, *P. suffruticosa* ssp. *spontanea* and *P. szechanica* have identical sequences of *psb*A–*trn*H intergenic spacer, and *P. rockii* and *P. szechanica* have identical *mat*K sequences.

Reticulate evolution in section Paeonia—Phylogenies of section *Paeonia* obtained from ITS and *mat*K sequences are concordant in certain respects and discordant in others (Figs. 1, 6). Both phylogenies support monophyly of each of the three sections of *Paeonia* as well as subsections of section *Moutan.* They differ substantially within section *Paeonia* (Fig. 4) where reticulate evolution has been documented previously by morphological, cytogenetic, and molecular data (Stebbins, 1948; Tzanoudakis, 1983; Sang, Crawford, and Stuessy, 1995). Discordance between the ITS and *mat*K phylogenies, therefore, may result from hybrid speciation followed by inheritance of cpDNA from one parent and fixation of the ITS sequences from another parent (Rieseberg and Soltis, 1991; Soltis and Kuzoff, 1995). Since maternal transmission of cpDNA has been found in the majority of flowering plants, the parent whose cpDNA is transmitted to hybrids is very likely the maternal parent (Corriveau and Coleman, 1988; Harris and Ingram, 1991; Mogensen, 1996). A synthesis of both gene phylogenies leads to a more refined hypothesis of species phylogeny reflecting both divergent and reticulate evolution (Fig. 7). Besides hybrid speciation suggested directly by ITS sequence ad-

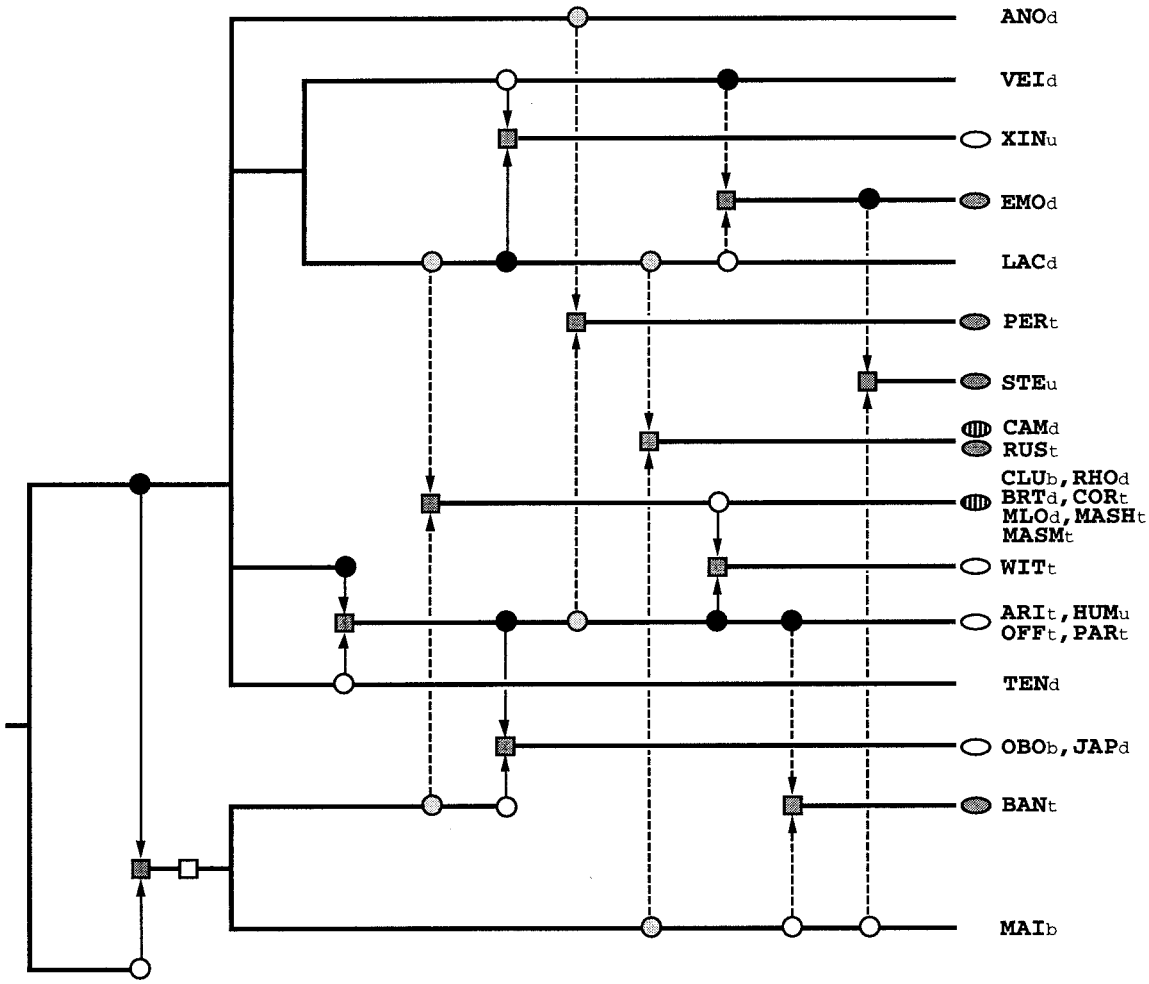

Fig. 7. Phylogeny of *Paeonia* section *Paeonia* reconstructed from a synthesis of the ITS and *mat*K phylogenies (Figs. 1, 6). Solid lines represent divergent and patristic evolution, but length of lines is not proportional to amount of patristic change. Dashed lines with arrows represent hybridization identified based on ITS sequence additivity (Sang, Crawford, and Stuessy, 1995); solid lines with arrows represent reticulate evolution determined based on discordances between the *mat*K and ITS phylogenies. Solid circles, parents from which cpDNA is inherited; open circles, parents from which ITS sequences are fixed; shaded circles, parents with uncertainty of above situations; shaded squares, hybrids; open square, a hybrid with fixed ITS sequences similar to one parent; open ellipse, hybrid species with fixed ITS sequences of one parent; shaded ellipse, hybrid species with fully additive ITS sequences from their parents; hatched ellipse, hybrid species with partially additive ITS seuqences from their parents. For species abbreviations, see Table 1. d = diploid; t = tetraploid; b = both diploid and tetraploid populations known; u = ploidy level unknown.

ditivity (Fig. 6), additional hybridization events are hypothesized by comparing distinct topological conflicts between the ITS and *mat*K phylogenies (Figs. 4, 7).

One difference between the two phylogenies is the absence in the *mat*K phylogeny of the two major basal clades of the ITS phylogeny (Figs. 1, 6). We suggest that the two ITS clades represent the early evolutionary split within section *Paeonia*. This was, then, followed by hybridization between the ancestor of the smaller clade (including *P. mairei, P. japonica,* and *P. obovata*) and an early evolutionary lineage of the larger ITS clade. It is more likely that the hybrid fixed the type of ITS sequences of the smaller clade, and inherited cpDNA from the larger clade given the same number of substitutions (seven) supporting the entire section on the *mat*K phylogeny and the larger clade on the ITS phylogeny. A comparable number of substitutions in ITS and *mat*K sequences is also found to support section *Oneapia* (five for ITS and six for *mat*K) and section *Moutan* (four for ITS and six for *mat*K) (Figs. 1, 6). The ancestral populations of the lineage represented by the smaller ITS clade, which might not have been involved in the hybridization, may have gone extinct. Therefore, two early divergent types of ITS sequences are maintained but one of the early divergent types of chloroplast genomes was lost after the hybridization.

The same explanation can be given to account for the hybrid origin of species *P. xinjiangensis, P. japonica, P. obovata, P. wittmanniana, P. arietina, P. humilis, P. officinalis,* and *P. parnassica,* which also have discordant positions in the two gene phylogenies (Figs. 1, 6, 7). *Paeonia xinjiangensis* forms a strongly supported sister group with *P. veitchii* on the ITS phylogeny (98% bootstrap value), but switches its sister group relationship to *P. lactiflora* on the *mat*K phylogeny, suggesting that *P. xinjiangensis* is a hybrid that fixed ITS sequences of *P. veitchii* and inherited cpDNA from *P. lactiflora.* Likewise, *P. japonica* and *P. obovata,* which are distantly

separated from four species (*P. arietina, P. humilis, P. officinalis,* and *P. parnassica*) on the ITS phylogeny, become the sister groups to them on the *mat*K phylogeny, indicating that *P. japonica* and *P. obovata* were derived from hybridization between the lineage containing these four species as the cpDNA donor and the other parent with the type of ITS sequences belonging to the smaller clade of the ITS phylogeny. By the same reasoning, the hybrid origin of *P. wittmanniana* is also suggested (Figs. 1, 6, 7).

Four species (*P. arietina, P. humilis, P. officinalis,* and *P. parnassica*) and *P. tenuifolia* form a strongly supported clade (100% bootstrap value) on the ITS phylogeny, but become two separate lineages on the *mat*K phylogeny. We suggest that the four species were derived through hybridization with *P. tenuifolia* serving as one parent. The other parent was either from an extinct basal lineage on the *mat*K phylogeny or it still cannot be identified by the present data. The hybrid species subsequently fixed ITS sequences of *P. tenuifolia* and thus becomes its sister group on the ITS phylogeny, but exists as an independent clade on the *mat*K phylogeny as did its cpDNA parent. This hypothesis is in agreement with the previous cytogenetic studies that revealed that *P. arietina, P. officinalis,* and *P. parnassica* are allotetraploids (Stebbins, 1948; Tzanoudakis, 1983; Schwarzacher-Robinson, 1986).

If a hybrid species inherited cpDNA and fixed ITS sequences from the same parent, however, the hybridization would not be detected by such a comparison. The present reconstruction, therefore, may still be an underestimate of reticulate evolution in *Paeonia.* Solutions to this problem include increase of intraspecific sample size (Doebley, 1989) and reconstruction of independent nuclear gene phylogenies. Nonetheless, the present reconstruction (Fig. 7) should represent more closely the species phylogeny than either ITS or *mat*K Phylogeny alone, and thus serve as a hypothesis to be tested with additional data.

An alternative explanation of discordance between nuclear and cpDNA phylogenies is lineage sorting (Doyle, 1992; Avise, 1994), but this seems less likely in the present case. One may hypothesize that the single basal clade of the *mat*K phylogeny is the true species phylogeny for section *Paeonia* and that the two basal clades of the ITS phylogeny result from random sorting of ancestral polymorphisms. It is very unlikely that two such different types of ITS sequences co-existed in the ancestral populations of section *Paeonia,* which would then have allowed lineage sorting. In other words, the lineage sorting hypothesis may require an extremely long coalescence time, which in turn requires very large effective population size (Pamilo and Nei, 1988; Kreitman, 1991; Hudson, 1992; Moore, 1995). It is, however, impossible to estimate the effective population size here because the divergence rate of ITS sequences is unkown in *Paeonia.* In any event, it seems that the longer the coalescence time required for the lineage sorting hypothesis, the more likely that hybridization may be involved, especially in some plant groups, such as peonies, where reticulate evolution coupled with polyploidization has been documented. In this context, it seems reasonable to invoke the hybridization hypothesis to explain the other distinct conflicts between the ITS and *mat*K phylogenies of section *Paeonia.* Statistical models that may help determine the significant levels of preference for hybridization vs. lineage sorting, however, are not available. Development of such models is much needed for accurate interpretation of conflicting gene phylogenies (Moore, 1995). Obtaining additional independent gene phylogenies can also test the hybridization hypothesis, and sequencing studies of low-copy nuclear genes of peonies are in progress.

Hybrid speciation at different ploidy levels—Allopolyploidy has been considered to be a primary mode for the formation of fertile and stable hybrid species (Grant, 1981). Frequency of natural hybrid speciation at the diploid level, however, has been controversial (Rieseberg, Carter, and Zona, 1990; Rieseberg, 1991; Wolfe and Elisens, 1994; Rieseberg, Van Fossen, and Desrochers, 1995). In section *Paeonia,* the species of hybrid origin identified by ITS and cpDNA sequences include six diploid and ten tetraploid species, and three species with both diploid and tetraploid populations (Fig. 7). The proportion of diploids among the hybrids species is unusually high, suggesting that hybrid speciation at the diploid level has been quite successful in peonies. An even more striking phenomenon is the co-existence of diploids and tetraploids in the same species or a group of species with the same origin (Fig. 7). For example, *Paeonia broteri* (diploid) and *P. coriacea* (tetraploid), which are endemic to southern Spain and northern Africa, may have the same origin because they share a substitution in the *mat*K phylogeny (Fig. 1). Extensive vegetative reproduction by rhizomes in peonies may have facilitated survival of initial diploid populations of hybrids until they became fertile or polyploidized. Existence of different ploidy levels in the same or very closely related species may eventually lead to reproductive isolation and further speciation. Studies of reproductive biology and the application of more sensitive molecular markers at the populational level are necessary for understanding hybrid speciation in *Paeonia.*

Molecular evolution in *P. cambessedesii* (diploid) and *P. russi* (tetraploid), two endemic species in the western Mediterranean islands, is noteworthy. ITS sequences of *P. russi* show nucleotide additivity at nine out of ten sites that are variable between *P. lactiflora* and *P. mairei,* strongly suggesting that *P. russi* is derived via hybridization between these two species. *Paeonia cambessedesii* shows only partial additivity at three of the variable sites, and was considered previously to have the same origin as a group of species with partial additivity of ITS sequences (Fig. 6; Sang, Crawford, and Stuessy, 1995). The *mat*K phylogeny supports the sister group relationship of *P. russi* and *P. cambessedesii,* and thus suggests the same origin for both species, i.e., derived through hybridization between *P. lactiflora* and *P. mairei.* The result further implies that gene conversion has operated more rapidly in diploid *P. cambessedesii* than in tetraploid *P. russi.* This is reasonable because in diploids, loci of nrDNA are more easily brought together during meiosis, which allows more effective interaction among these loci and thus more rapid gene conversion (Arnheim, 1983). However, this hypothesis does not apply to a group of diploid and tetraploid species, *P. clusii, P. rhodia, P. broteri, P. co-*

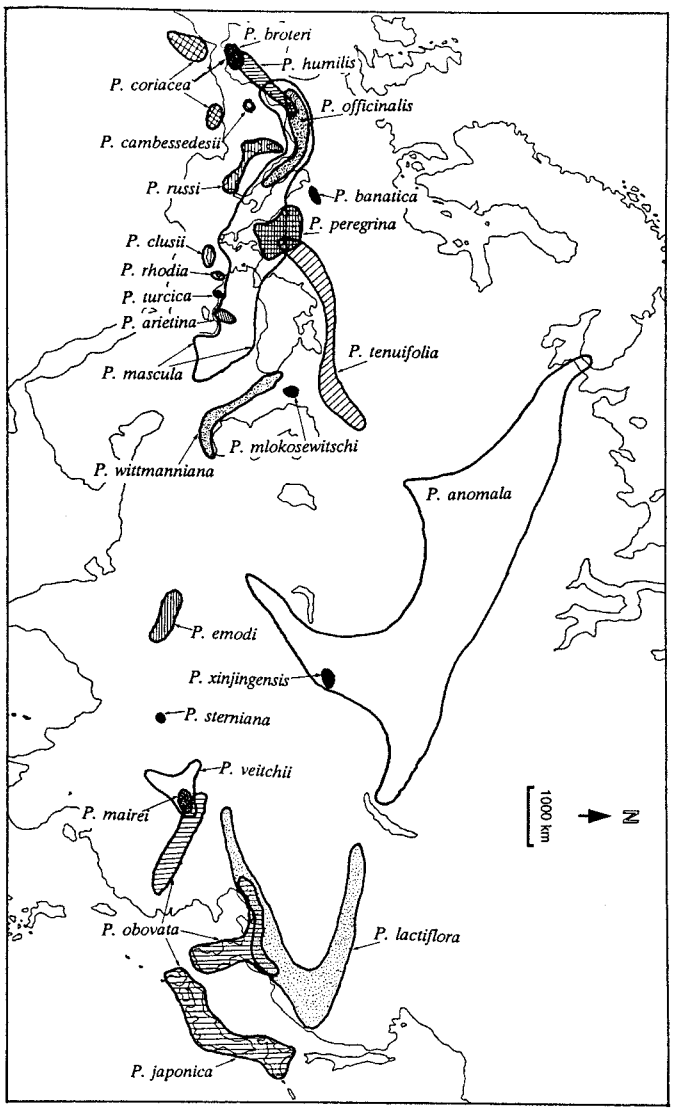

Fig. 8. Distribution of species of *Paeonia* section *Paeonia* in Eurasia.

riacea, P. mlokosewischi, and two *P. mascula* subspecies, which may have the same origin and almost identical ITS sequences. In particular, diploid *P. broteri* and tetraploid *P. coriacea,* whose common origin is supported by one substitution in *mat*K (Fig. 1), have the same pattern of partial additivity of ITS sequences. The tempo of concerted evolution at different ploidy levels, thus, needs further investigation.

Biogeography—An intercontinental disjunction occurs between section *Oneapia,* endemic to western North America and the other two sections found in Eurasia. The isolation could have resulted either from a vicariance event disrupting continuous distribution of the ancestral populations between Eurasia and western North America, or a long-distance dispersal from one region to the other. The vicariance explanation is favored here because *Paeonia,* with follicle fruits and seeds having smooth surfaces and diameters of 7–13 mm, does not appear to have great dispersal ability. Continuous distribution of ancestral populations of *Paeonia* between Eurasia and western North America is likely to have existed through the Bering land bridge, which allowed periodical exchange of temperate plants between eastern Asia and western North America until late Tertiary or Quaternary (Wolfe, 1975, 1980; Tiffney, 1985). Disruption of this continuous distribution may have been due to climatic cooling at high latitudes and/or submergence of the Bering land bridge (Tiffney, 1985).

The time of such a vicariance event can be estimated using a molecular clock. Time of divergence may be calculated as the value of DNA sequence divergence divided by twice the sequence divergence rate. For peonies, sequence divergence rates of the sequenced DNA regions are unknown, and cannot be estimated by either fossil records or biogeographic events. We can only use rates estimated in other plant groups. ITS sequences are probably not a good choice for use as a molecular clock because the rates of ITS sequences calculated in several plant groups vary considerably (Suh et al., 1993; Sang et al., 1995). For *mat*K sequences, the overall divergence rates were suggested as being approximately twice as fast as that of *rbc*L sequences (Steele and Vilgalys, 1994). If an average overall divergence rate of 2×10^{-10} per site per year is used for *rbc*L sequences (Albert et al., 1994), a rate of 4×10^{-10} per site per year can be used for *mat*K sequences. The divergence time between section *Oneapia* and the rest of the genus, therefore, is estimated to be 16.6 million years ago (mya). This estimate, however, is subject to several sources of error. First, the divergence rate of *rbc*L may not apply to peonies, because it can vary in different groups with different generation times (Li, Tanimura, and Sharp, 1987; Clegg, 1990; Gaut et al., 1992). Vegetative reproduction by rhizomes is very common in peonies, which may significantly prolong generation time, and consequently yield slower rates of DNA divergence. In this case, the divergence time may be underestimated. Second, the estimation that *mat*K evolves twice as fast as *rbc*L is rough and may actually be different in peonies. Nevertheless, although estimation of divergence rates or times using the molecular clock hypothesis has been based largely on uncertain assumptions and approximate values, it continues to be useful in helping understand tempos of evolution and plant historical biogeography (Parks and Wendel, 1990; Crawford, Lee, and Stuessy, 1992; Wendel and Albert, 1992).

The estimated time for formation of the intercontinental disjunction in peonies, 16.6 mya, is middle Miocene. Tiffney (1985) suggested that during the Miocene, temperatures at higher latitude allowed exchange of deciduous temperate plants via the Bering land bridge. Further, many herbaceous angiosperm groups evolved during the Miocene and exhibited an eastern Asian–eastern North American disjunct distribution (Tiffney, 1985). Formation of intercontinental disjunction in peonies, therefore, may well be a result of disruption of continuous distribution through the Bering land bridge during Miocene time. The possible existence of ancestral populations of extant *Paeonia* species in high latitudes around the Bering land bridge in the Miocene is concordant with warm climate during this period (Potts and Behrensmeyer, 1992).

Distributional histories of the largest and most widespread section *Paeonia* may have been much confounded by Pleistocene glaciation (Fig. 8). Reconstruction of complex reticulate evolution within the section provided essential evidence for understanding its biogeography (Sang, Crawford, and Stuessy, 1995). It is striking that after hybridization, European populations of the present Asian species appear to have been completely replaced by their hybrids. Extensive hybridization of peony species must have produced a wide spectrum of different genome combinations upon which natural selection could act (Rieseberg and Wendel, 1993; Arnold and Hodges, 1995). Hybrids that adapted to drastic climatic changes during Pleistocene in Europe are currently distributed in the Mediterranean region. Asian species did not survive such changes in Europe, and their distributions became more restricted. The hybrid species *P. xinjiangensis*, *P. emodi*, and *P. sterniana* may represent footprints of the eastern Asian species *P. lactiflora*, *P. veitchii*, and *P. mairei* when their distributional ranges became reduced to only eastern Asia (Fig. 8). Eastern Asia was much less seriously affected by Pleistocene glaciation and may have provided refugia for the nonhybrid peony species (Potts and Behrensmeyer, 1992; Tao, 1992).

In the Mediterranean region, effects of periodical glaciation on distributions of peony species are speculated. A group of species, *P. clusii*, *P. rhodia*, *P. broteri*, *P. coriacea*, *P. mlokosewischi*, and *P. mascula* ssp. hellenica and ssp. mascula, endemic to widely different areas in the Caucasus and throughout the southern Mediterranean, may have had a single hybrid origin (Figs. 7, 8). Other than the hypothesis that these hybrid species were dispersed widely across the southern Mediterranean region, a vicariance explanation is again favored. After hybridization, the ancestral hybrid populations might have migrated northward and extended their distributional ranges both eastward and westward during a glacial interval. During subsequent glaciation, these populations were forced into isolated areas in the southern Mediterranean region. Geographic isolation consequently led to speciation among these populations and created the complex diversity now seen for this group of species. A similar distributional pattern is found in another group of Mediterranean species, *P. arietina*, *P. humilis*, *P. officinalis*, and *P. parnassica*, which also have the same hybrid origin.

LITERATURE CITED

ALBERT, V. A., A. BACKLUND, K. BREMER, M. W. CHASE, J. R. MANHART, B. D. MICHLER, AND K. NIXON. 1994. Functional constraints and *rbc*L evidence for land plant phylogeny. *Annals of the Missouri Botanical Garden* 81: 534–567.

ALDRICH, J., B. W. CRNHEEY, E. MERLIN, AND L. CHRISTOPHERSON. 1988. The role of insertions/deletions in the evolution of the intergenic region between *psb*A and *trn*H in the chloroplast genome. *Current Genetics* 14: 137–146.

ARNHEIM, N. 1983. Concerted evolution of multigene families. *In* M. Nei and R. K. Koehn [eds.], Evolution of gene and protein, 38–61. Sinauer, Sunderland, MA.

ARNOLD, M. L., AND S. A. HODGES. 1995. Are natural hybrids fit or unfit relative to their parents? *Trends in Ecology and Evolution* 10: 67–71.

AVISE, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York, NY.

BARBER, H. N. 1941. Evolution in the genus *Paeonia*. Nature 148: 227–228.

BOHLE, U.-R., H. HILGER, R. CERFF, AND W. F. MARTIN. 1994. Noncoding chloroplast DNA for plant molecular systematics at the infrageneric level. *In* B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle [eds.], Molecular ecology and evolution: approaches and applications, 391–403. Birkhauser Verlag, Basel.

CLEGG, M. T. 1990. Dating the monocot–dicot divergence. *Trends in Ecology and Evolution* 5: 1–2.

———, B. S. GAUT, G. H. LEARN, JR., AND B. R. MORTON. 1994. Rates and patterns of chloroplast DNA evolution. *Proceedings of the National Academy of Sciences, USA* 91: 6795–6801.

CORRIVEAU, J. L., AND A. W. COLEMAN. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *American Journal of Botany* 75: 1443–1458.

CRAWFORD, D. J., N. S. LEE, AND T. F. STUESSY. 1992. Plant species disjunctions: perspectives from molecular data. *Aliso* 13: 395–409.

DOEBLEY, J. F. 1989. Molecular evidence for a missing wild relative of maize and the introgression of its chloroplast genome into *Zea perennis*. *Evolution* 43: 1555–1558.

DOYLE, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany* 17: 144–163.

———, J. I. DAVIS, R. J. SORENG, D. GARVIN, AND M. J. ANDERSON. 1992. Chloroplast DNA inversions and the origin of the grass family (Poaceae). *Proceedings of the National Academy of Sciences, USA* 89: 7722–7726.

———, AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

FANG, W. 1958. Notes on Chinese peonies. *Acta Phytotaxonomica Sinica* 7: 288–323.

FELSENSTEIN, J. 1985. Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution* 39: 783–791.

———. 1994. PHYLIP: phylogenetic inference package, version 3.5c. University of Washington, Seattle, WA.

GAMBRILL, K. W. 1988. King of flowers, queen of herbs: the peony. *Pacific Horticuture* 49:26–32.

GAUT, B. S., S. V. MUSE, W. D. CLARK, AND M. T. CLEGG. 1992. Relative rates of nucleotide substitution at the *rbc*L locus of monocotyledonous plants. *Journal of Molecular Evolution.* 35: 292–303.

GIELLY, L., AND P. TABERLET. 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbc*L sequences. *Molecular Biology and Evolution* 11: 769–777.

GOLENBERG, E. M., M. T. CLEGG, M. L. DURBIN, J. DOEBLEY, AND D. P. MA. 1993. Evolution of a noncoding region of the chloroplast genome. *Molecular Phylogenetics and Evolution* 2: 52–64.

GRANT, V. 1981. Plant speciation, 2d ed., 191–353. Columbia University Press, New York, NY.

HAM, R. C. H. J., H. 'T. HART, T. H. M. MES, AND J. M. SANDBRINK. 1994. Molecular evolution of noncoding regions of the chloroplast genome in the Crassulaceae and related species. *Current Genetics* 25: 558–566.

HARRIS, P. A., AND R. INGRAM. 1991. Chloroplast DNA and biosystematics: the effects of intraspecific diversity and plastid transmission. *Taxon* 40: 393–412.

HONG, D., Z. ZHANG, AND X. ZHU. 1988. Studies on the genus *Paeonia* (1)—report of karyotypes of some wild species in China. *Acta Phytotaxonomica Sinica* 26: 33–43.

HUDSON, R. R. 1992. Gene trees, species trees, and segregation of ancestral alleles. *Genetics* 131: 509–512.

JANSEN, R. K., AND J. D. PALMER. 1987. A chloroplast DNA inversion marks an ancient evolutionary split in the sunflower family (Asteraceae). *Proceedings of the National Academy of Sciences, USA* 84: 5818–5822.

JOHNSON, L. A., AND D. E. SOLTIS. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxifragaceae sensu stricto. *Systematic Botany* 19: 143–156.

JUKES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. *In* H. N. Munro [ed.], Mammalian protein metabolism, 21–132. Academic Press, New York, NY.

KREITMAN, M. 1991. Detecting selection at the level of DNA. In R. K.

Selander, A. G. Clark, and T. S. Whittam [eds.], Evolution at the molecular level, 204–221. Sinauer, Sunderland, MA.

LARSON, A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. *In* B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle [eds.], Molecular ecology and evolution: approaches and applications, 371–390. Birkhauser Verlag, Basel.

LEVINSON, G., AND G. A. GUTMAN. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Molecular Biology and Evolution* 4: 203–221.

LI, W.-H., M. TANIMURA, AND P. M. SHARP. 1987. An evaluation of the molecular clock hypothesis using mammalian DNA sequences. *Journal of Molecular Evolution* 25: 330–342.

MADDISON, W. P., M. J. DONOGHUE, AND D. R. MADDISON. 1984. Outgroup analysis and parsimony. *Systematic Zoology* 33: 83–103.

MANER, J.-F., A. NATALI, AND F. EHRENDORFER. 1994. Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergene region. *Plant Systematics and Evolution* 190: 195–212.

MASON-GAMER, R., AND E. A. KELLOGG. In press. Testing for phylogenetic conflict among molecular data sets in the tribe triticeae (Gramineae). *Systematic Biology*.

MES, T. H. M., AND H. 'T. HART. 1994. *Sedum surculosum* and *S. jaccardianum* (Crassulaceae) share a unique 70 bp deletion in the chloroplast DNA *trn*L(UAA)–*trn*F(GAA) intergenic spacer. *Plant Systematics and Evolution* 193: 213–221.

MOGENSEN, H. L. 1996. The hows and whys of cytoplasmic inheritance in seed plants. *American Journal of Botany* 83: 383–404.

MOORE, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* 49: 718–726.

MORTON, B. R., AND M. T. CLEGG. 1993. A chloroplast DNA mutational hot spot and gene conversion in a noncoding region near *rbc*L in the grass family (Poaceae). *Current Genetics* 24: 357–365.

NEUHAUS, H., AND G. LINK. 1987. The chloroplast *trn*Atys(UUU) gene from mustard (*Sinapsis alba*) contains a class II intron potentially coding for a maturase-related polypeptide. *Current Genetics* 11: 251–257.

NOONAN, G. R. 1988. Biogeography of North American and Mexican insects, and a critique of vicariance biogeography. *Systematic Zoology* 37: 366–384.

OLMSTEAD, R. G., AND J. D. PALMER. 1994. Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* 81: 1205–1224.

PAMILO, P., AND M. NEI. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* 5: 568–583.

PAN, K.-Y. 1979. *Paeonia.* In Flora reipublicae siniccae, vol. 27, 37–59. Science Press, Beijing.

PARKS, C. R., AND J. F. WENDEL. 1990. Molecular divergence between Asian and North American species of *Liriodendron* (Magnoliaceae) with implications for interpretation of fossil floras. *American Journal of Botany* 77: 1243–1256.

PEI, Y.-L. 1993. Studies on the *Paeonia suffruticosa* Andr. Complex. Ph.D. dissertation. Institute of Botany, Chinese Academy of Sciences, Beijing.

POTTS, R., AND A. K. BEHRENSMEYER. 1992. Late Cenozoic Terrestrial ecosystems. *In* A. K. Behrensmeyer, J. D. Damuth, W. A. Dimichele, R. Potts, H.-D. Sues, and S. L. Wing [eds], Terrestrial ecosystems through time, 419–542, University of Chicago Press, Chicago, IL.

RAUBERSON, L. A., AND R. K. JANSEN. 1992. Chloroplast DNA evidence on the ancient evolutionary split in vascular land plants. *Science* 255: 1697–1699.

RIESEBERG, L. H. 1991. Homoploid reticulate evolution in *Helianthus*: evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

———, R. CARTER, AND S. ZONA. 1990. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). *Evolution* 44: 1498–1511.

———, AND D. E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.

———, AND C. VAN FOSSEN, AND A. DESROCHERS. 1995. Genomic reorganization accompanies hybrid speciation in wild sunflowers. *Nature* 375: 313–316.

———, AND J. F. WENDEL. 1993. Introgression and its consequences in plants. *In* R. G. Harrison [ed.], Hybrid zone and the evolutionary process, 70–109. Oxford University Press, New York, NY.

SANG, T. 1995. Phylogeny and biogeography of *Paeonia* (Paeoniaceae). Ph.D. dissertation, Ohio State University, Columbus, OH.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using sequences of internal transcribed spacer of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences, USA* 92: 6813–6817.

———, D. J. CRAWFORD, T. F. STUESSY, AND M. O. SILVA. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany* 20: 55–64.

SCHWARZACHER-ROBINSON, T. 1986. Meiosis, SC-formation, and karyotype structure in diploid *Paeonia tenuifolia* and tetraploid *P. officinalis. Plant Systematics and Evolution* 154: 259–274.

SHINOZAKI, K., ET AL. 1986. The complete nucleotide sequence of the tobacco chloroplast genome. *Plant Molecular Biology Report* 4: 110–147.

SOLTIS, D. E., AND R. K. KUZOFF. 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). *Evolution* 49: 727–742.

STEBBINS, G. L., JR. 1938a. Notes on the systematic relationships of the Old World species and of some horticultural forms of the genus *Paeonia. University of California Publications in Botany* 19: 245–266.

———. 1938b. The western American species of *Paeonia*. *Madroño* 4: 252–260.

———. 1948. Review of ''A study of the genus *Paeonia* by F. C. Stern.'' *Madrono* 9: 193–199.

———, AND S. ELLERTON. 1939. Structural hybridity in *Paeonia californica* and *P. brownii*. *Journal of Genetics* 38: 1–36.

STEELE, K. P., AND R. VILGALYS. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. *Systematic Botany* 19: 126–142.

STERN, F. C. 1946. A study of the genus *Paeonia*. Royal Horticultural Society, London.

STREISINGER, G., AND J. OWEN. 1985. Mechanisms of spontaneous and induced frameshift mutation in bacteriophage T4. *Genetics* 109: 633–659.

SUH, Y., L. B. THUEH, H. E. REEVE, AND E. A. ZIMMER. 1993. Molecular evolution and phylogenetic implication of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *American Journal of Botany* 80: 1042–1055.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign, IL.

SYTSMA, K. J., AND J. F. SMITH. 1992. Molecular systematics of Onagraceae: examples from *Clarkia* and *Fuchia.* In P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds], Molecular systematics of plants, 295–323. Chapman and Hall, New York, NY.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TAKHTAJAN, A. 1969. Flowering plants. origin and dispersal. Oliver & Boyd, Edinburgh.

———. 1987. Systema Magnoliophytorum, 54–55. Officina Editoria ''Nauka'', Leninopoli.

TAO, J. 1992. The Tertiary vegetation and flora and floristic regions in China. *Acta Phytotaxonomica Sinica* 30: 25–43.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endoclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evoution* 37: 221–244.

THORNE, R. F. 1992. Classification and geography of the flowering plants. *Botanical Review* 58: 225–348.

TIFFNEY, B. H. 1985. Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *Journal of Arnold Arboretum* 65: 73–94.

TZANOUDAKIS, D. 1983. Karyotypes of four wild *Paeonia* species from Greece. *Nordic Journal of Botany* 3: 307–318.

WATROUS, L. E., AND Q. D. WHEELER. 1981. The out-group comparison method of character analysis. *Systematic Zoology* 30: 1–11.

WENDEL, J. F., AND V. A. ALBERT. 1992. Phylogenetics of the cotton genus (*Gossypium*): character-state weighted parsimony analysis of

chloroplast-DNA restriction site data and its systematic and biogeographic implications. *Systematic Botany* 17: 115–143.

———, A. SCHNABEL, AND T. SEELANAN. 1995. Bi-directional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proceedings of the National Academy of Sciences, USA* 92: 280–284.

WOLFE, A. D., AND W. J. ELISENS. 1994. Nuclear ribosomal DNA restriction-site variation in *Penstemon* section *Peltanthera* (Scrophulariaceae): an evaluation of diploid hybrid speciation and evidence for introgression. *American Journal of Botany* 81: 1627–1635.

———, AND ———. 1995. Evidence of chloroplast capture and pollen-mediated gene flow in *Penstemon* section *Peltanthera* (Scrophulariaceae). *Systematic Botany* 20: 395–412.

WOLFE, J. A. 1975. Some aspects of plant geography of the Northern Hemisphere during the late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* 62: 264–279.

———. 1980. Tertiary climatic and floristic relationships at high latitudes in the Northern Hemisphere. *Paleogeography, Plaeoclmatics, and Paleoecology* 61: 33–77.

WOLFSON, R., K. G. HIGGINS, AND B. B. SEARS. 1991. Evidence for replication slippage in the evolution of *Oenothera* chloroplast DNA. *Molecular Biology and Evolution* 8: 709–720.