

CHLOROPLAST DNA VARIATION IN *POLYGONATAE* *SENSU LATO* (LILIACEAE)

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Abstract

cp DNA variations were examined in 16 species of 7 genera of the tribe *Polygonatae* for 50 probe-enzyme combinations to study phylogenetic relationship among genera and species. The shared fragments were counted, index of similarity and distance matrix calculated. The study supports the idea against monophyly of *Polygonatae sensu lato*. The results suggest that there are three monophyletic groups within *Polygonatae sensu lato*. Group I with genera *Maianthemum*, *Smilacina* and *Disporopsis*; group II with *Uvularia* and *Disporum* and group III with genera *Prosartes* and *Clintonia*. The differences between each group (estimated F value \times 100) were 5.88-7.8 for group I and II, 4.8-6.6 for I and III and 3.4-5.61 for II and III. The distance within the genus *Prosartes* was 0.33-0.84, while it differed from *Disporum* in 3.4-5.52. The difference of *Prosartes* from *Clintonia* was 2.2, while between *Disporum* and *Uvularia* as 1.63-0.171. The RFLP studies turned out to be concordant to morphological data. The present findings support lumping of *Smilacina* in *Maianthemum*.

The results support recent morphological, karyological and molecular arguments of restoration of genus *Prosartes* as an independent genus. The results also indicate that *Uvularia* is closely related to *Disporum* while *Clintonia* is closer to the genus *Prosartes*.

Introduction

Phylogenetic analysis of the tribe *Polygonatae* is complicated by different taxonomic perspective regarding composition and internal organization of the group. The generic and intergeneric taxonomy of Liliaceae-*Polygonatae* has been controversial. Bentham & Hooker (1883) placed some genera from 3 different tribes of family Asparagaceae of Baker (1875) in Liliaceae-*Polygonatae*. Krause (1930) expanded the tribe by adding *Clintonia*, *Disporum* on the basis of similarity in the characters of ovary followed by the reports of Melchior (1964), Sen (1975) and Thorne (1983).

The higher order taxonomy of the tribe is also quite complicated. Starting from Liliales-Asparagaceae-*Polygonatae* (Baker, 1875), through Liliaceae-*Polygonatae* (Krause, 1930; Hutchinson, 1934; Thorne, 1983; Sen, 1975) to Asparagales-Convallariaceae-*Polygonatae* (Dahlgren *et al.*, 1985; Takhtajan 1987). Dahlgren *et al.*, (1985) were of the view that genera like *Disporum*, *Streptopus* and *Clintonia* are misplaced in the family Convallariaceae, perhaps due to convergence of embryological and some morphological characters. The generic and intergeneric taxonomy of *Disporum* and *Prosartes*, *Maianthemum* and *Smilacina* has also been controversial (Conover, 1983, 1991; LaFrankie, 1986; Hara, 1987, 1988; Heng, 1990; Hong & Zhu, 1990, Shinwari, 1994a,b,c; Fukuhara & Shinwari, 1994; Shinwari, 1995, 1998).

In an attempt to revise the phylogenetic relationship among genera of *Polygonatae* RFLP studies were carried out in 16 species of seven genera of the tribe.

Table 1. Sources of plant materials.

Species	Localities	Collector(s)	* Accession No.
<i>Disporum</i> D. Don	Japan: Kyoto Pref.: Ohmiya-cho, Mt. Takano	Z. K. Shinwari	1
<i>D. sessile</i> D. Don	Taiwan: Ilan Hsien: Mt. Tai-Ping	M. N. Tamura	2
<i>D. taipingense</i> Tamura et Kawano			
<i>Prosartes</i> D. Don			
<i>P. hookeri</i> Torrey	USA: Pennsylvania: Somerset Co., Powder mill	S. Kawano <i>et al.</i> ,	3
<i>P. trachycarpa</i> Watson	USA: Pennsylvania: Somerset Co., Powder mill	S. Kawano <i>et al.</i> ,	4
<i>P. maculata</i> (Buckley) Gray	USA: Ohio: Scioto Co., Shewnee st. Forests	S. Kawano <i>et al.</i> ,	5
<i>P. lanuginosa</i> (Mich.) Don	USA: Ohio: Scioto Co., Lump Black Run	S. Kawano <i>et al.</i> ,	6
<i>Uvularia grandiflora</i> Linn.	USA: Wisconsin: Marathen Co. Forest	S. Kawano <i>et al.</i> ,	7
<i>U. sessilifolia</i> Linn.	USA: Pennsylvania: Somerset Co., Powder mill	S. Kawano <i>et al.</i> ,	
<i>Disporopsis alisanensis</i>	Taiwan: Allisan	M. N. Tamura	9
<i>Smilacina trifolia</i>	USA: California: Mendocino Co.; Fisch Rock Road	S. Kawano <i>et al.</i> ,	10
<i>S. stellata</i>	USA: California: Santa Clara Co.: Mile Marker 4.5	S. Kawano <i>et al.</i> ,	11
<i>S. yezoensis</i>	Japan: Toyama Pref.: Mt. Tateyama	S. Kawano	12
<i>S. honoensis</i>	Japan: Nara Pref.: Mt. Wasamatta	Z. K. Shinwari	13
<i>S. racemosa</i>	USA: California: Del Norte Co.: Six River Nat. Forest	S. Kawano <i>et al.</i> ,	14
<i>Maianthemum dilatatum</i>	Japan: Hokkaido Pref.: Mt. Korri	M. N. Tamura	15
<i>Clintonia udensis</i>	Japan:	Z. K. Shinwari	16

*These numbers are used in Fig. 3.

Materials and Methods

Plant Materials: Eleven accessions of five different genera of tribe *Polygonatae sensu lato* were selected to represent 16 species. The sources of plant materials are summarized in Table 1. Voucher specimens have been deposited in the Herbarium of Department of Botany, Faculty of Science, Kyoto University, Japan (KYO).

DNA extraction: DNA from the leaf samples were extracted according to modified method of Tai & Tanksley (1990). Three to four grams of fresh leaves were snap frozen with liquid-nitrogen and crushed into fine powder with mortar and pestle. To the powdered leaves which were transferred into 50ml polypropyrene corning tube, 16ml of pre warmed extraction buffer (100mM Tris-HCl, pH 8.0; 50mM EDTA, pH 8.0; 50mM NaCl, 1.25% SDS; 10mM β -mercaptoethanol) was added. After a thorough mixing, the tube was incubated at 65°C for 10 min. Subsequently, 6 ml of 5M KAc was added to the tube, which was kept on ice for 20 min. Approximately 10 ml chloroform was added to each tube. After thorough mixing, the tube was centrifuged in Hitachi refrigerated centrifuge (20 PR) at 7,000 rpm for 10 min. The aqueous phase was transferred into a new tube and the DNA was precipitated by adding 12 ml of isopropanol and centrifuged at 7000 rpm for 8 min. The pellet was then dissolved in 600 μ l-TE, 30 mg RNase and was incubated at 65°C for 30 min. The insoluble debris was spun down in a microfuge at 15,000 rpm for 20 min. The supernatant was stored at 4°C.

Further purification: Crude DNAs were further purified by CsCl density gradient ultracentrifugation. Typically 50 μ g to 20 μ g of pure high-molecular DNA were obtained from 3 g of leaf tissue.

RFLP analysis: DNAs were digested with 12 six-base cutters (*Bam* HI; *Bgl* II; *DRA* I; *Eco* RI; *Eco* RV; *Hin* dIII; *Kpn* I; *Pst* I; *Pvu* II; *Sac* I; *Sca* I and *Xho* I), electrophoresed in agarose gels, transferred onto nylon membranes (Hybond-N, Amersham), and hybridized with *Dioscorea* probe (Terauchi *et al.*, 1989). The labelling of probes and detection of hybridization were done with a non-radioactive system using DIG-dutp kit (Boehringer Mannheim).

Phylogenetic trees: The maximum parsimony tree (PAUP, Swofford 1993) was constructed showing restriction fragment pattern similarity among these 16 taxa (Fig. 2). *Tricyrtis affinis* was used as an outgroup.

Results

RFLP analysis: Hybridization of the five cpDNA probes to southern blot with digest of 16 DNA samples for the 12 enzymes were examined. Small fragments (< 1 kb) predicted to occur could not be detected because of a restriction site loss/gain. Failure to detect these fragments most likely results from insufficient sensitivity of the southern hybridization to detect small fragments. An example of RFLP is shown in Fig. 1.

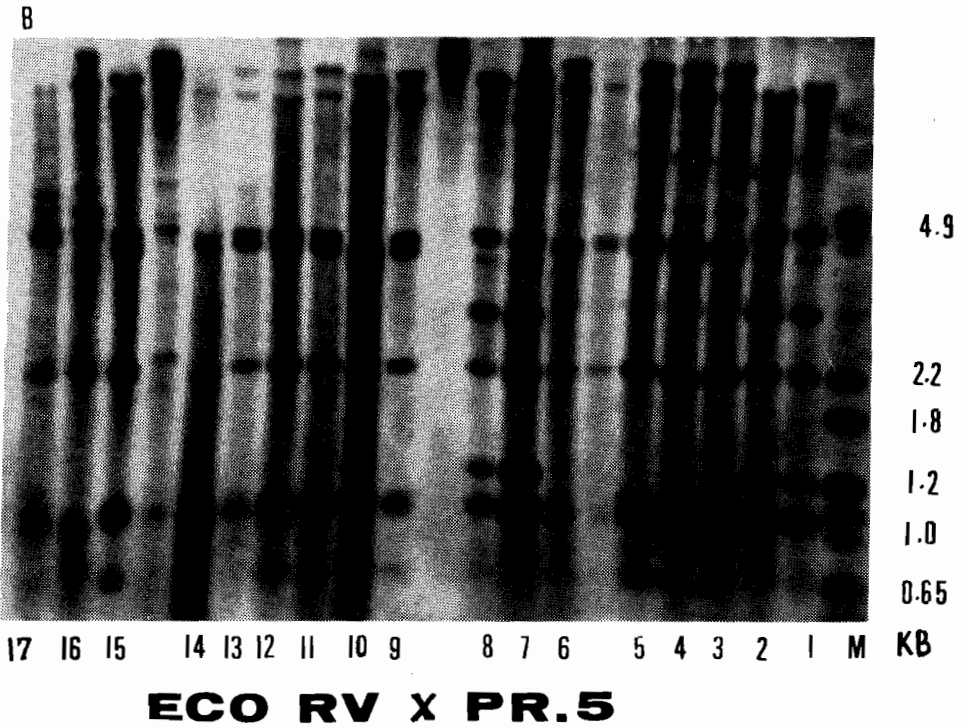
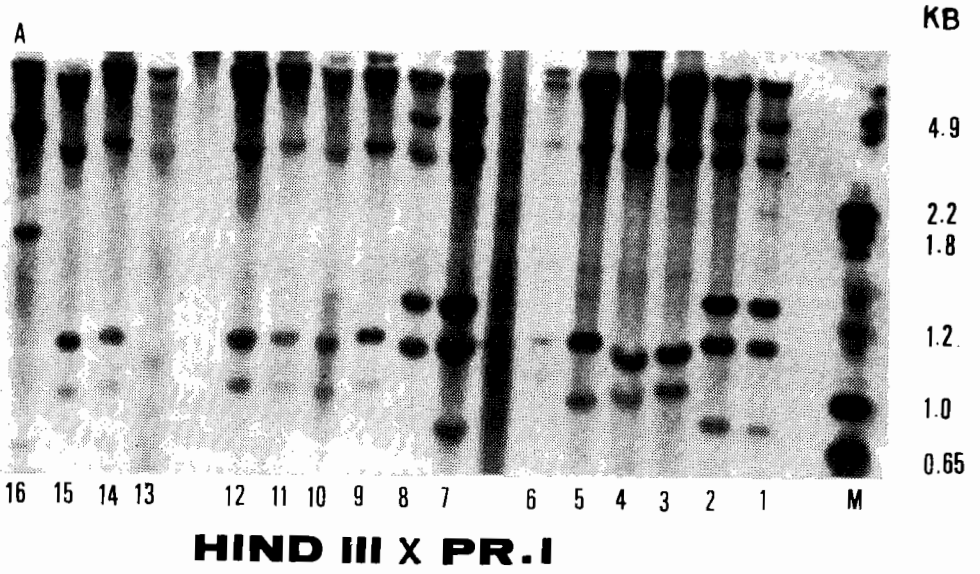


Fig. 1a. Hmd III x Pr.1, Fig. 1b. Eco RV x Pr.5

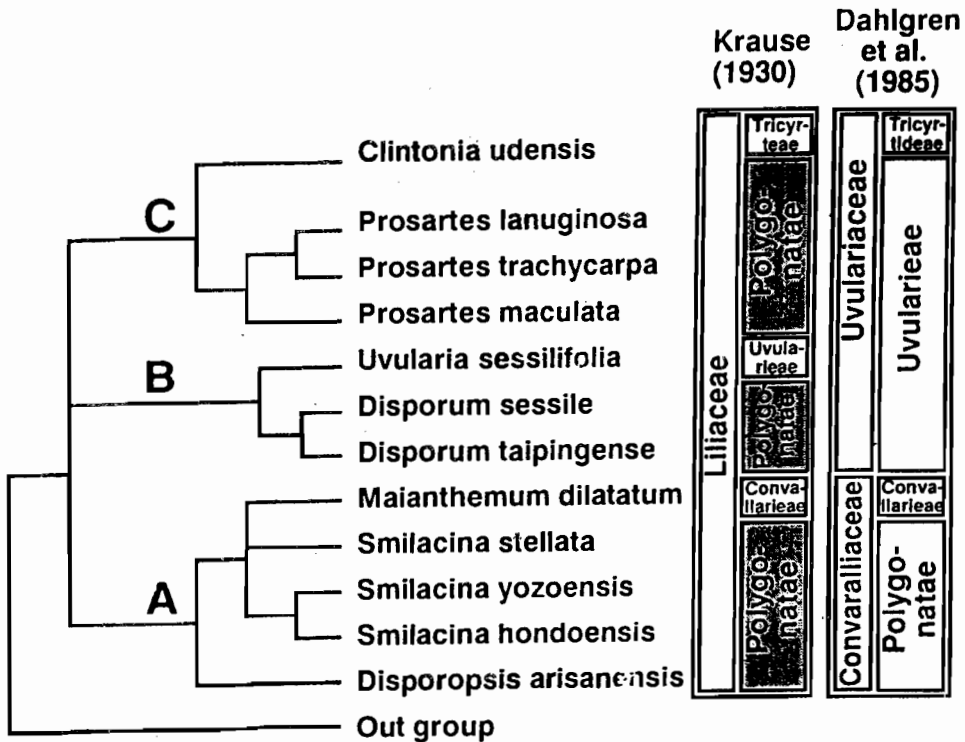


Fig. 2 Phylogenetic tree obtained by the Paup method. *Prosartes hookeri* and *Uvularia grandiflora* nested at the same position as *Prosartes trachycarpa* and *Uvularia sessilifolia*, respectively. Moreover, *Smilacina trifolia* and *S. racemosa* showed same position as that of *S. stellata*. Hence they are avoided in this figure.

The results suggest that there are three monophyletic groups within *Polygonatae sensu lato*. Group II with genera *Maianthemum*, *Smilacina* and *Disporopsis*; group III with *Uvularia* and *Disporum* and group I with genera *Prosartes* and *Clintonia*.

The result obtained by counting shared fragments and calculating the index of similarity and distance matrix is given in Table 2. The differences between each group were 5.88-7.8 for group I and III, 4.8-6.6 for I and III and 3.4-5.61 for II and III. The distance within the genus *Prosartes* was 0.33-0.84, while it differed from *Disporum* in 3.4-5.52. The difference of *Prosartes* from *Clintonia* was 2.2, while between *Disporum* and *Uvularia* as 1.63-0.171.

The data was examined using PAUP method. The divergence between *Disporum* and *Prosartes* was greater than that between *Disporum* and *Uvularia* or *Prosartes* and *Clintonia*. Within the genus *Prosartes*, *P. maculata* was different from the rest of the *Prosartes* species (Fig. 2).

In *rbcl* gene sequence data the base pair difference ranged from 6 (100d=0.44) to 114 (100d=9.03). Especially small values were observed between *Disporum sessile* and *D. taipingense* {6 (100d=0.44)}, *Smilacina hondoensis* and *Maianthemum dilatatum* {11 (100d=0.82)}, *Prosartes lanuginosa* and *P. maculata* (12(100d=0.88)). The taxa within group I (Fig. 1) differed in 100d=0.82-2.99, group III in 100d=0.44-2.16, and

Table 2. Distance matrix of sixteen taxa of Polygonatae.

Species Name	*1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Disporum sessile</i>	0.00	0.34	0.342	0.342	0.54	0.55	0.164	0.171	0.6	0.71	0.698	0.655	0.68	0.72	0.69	0.555
<i>D. taipingensis</i>			0.34	0.341	0.48	0.54	0.163	0.17	0.588	0.7	0.68	0.65	0.66	0.7	0.67	0.55
<i>Prosartes hookeri</i>				0.083	0.073	0.084	0.56	0.561	0.527	0.577	0.58	0.584	0.59	0.57	0.49	0.21
<i>P. trachycarpa</i>					0.033	0.034	0.502	0.502	0.561	0.576	0.579	0.6	0.591	0.6	0.48	0.22
<i>P. maculata</i>						0.034	0.5	0.501	0.511	0.58	0.58	0.6	0.6	0.57	0.5	0.21
<i>P. lanuginosa</i>							0.502	0.502	0.554	0.578	0.576	0.59	0.5	0.59	0.49	0.21
<i>Uvularia grandiflora</i>								0.042	0.706	0.76	0.759	0.761	0.77	0.76	0.68	0.63
<i>U. sessilifolia</i>									0.7	0.76	0.76	0.759	0.78	0.75	0.7	0.62
<i>Disporopsis arisanensis</i>										0.06	0.059	0.06	0.058	0.07	0.08	0.52
<i>Smilacina trifolia</i>											0.04	0.039	0.04	0.04	0.04	0.63
<i>S. stellata</i>												0.04	0.04	0.04	0.05	0.65
<i>S. yozoensis</i>													0.03	0.04	0.04	0.66
<i>S. hondoensis</i>														0.04	0.04	0.62
<i>S. racemosa</i>															0.45	0.63
<i>Maianthemum dilatatum</i>																0.57
<i>Clintonia udensis</i>																0.0

*1 = *Disprum sessile*, 2 = *D. taipingensis*, 3 = *Prosartes hookeri*, 4 = *P. trachycarpa*, 5 = *P. maculata*, 6 = *P. lanuginosa*, 7 = *Uvularia grandiflora*, 8 = *U. sessilifolia*, 9 = *Disporopsis arisanensis*, 10 = *Smilacina trifolia*, 11 = *S. stellata*, 12 = *S. yozoensis*, 13 = *S. hondoensis*, 14 = *S. racemosa*, 15 = *Maianthemum dilatatum*, 16 = *Clintonia udensis*.

group III in $100d=0.88-3.14$. Group I differed from group II and III in $100d= 6.99-9.03$ and $100d= 4.92-7.35$, respectively. Group II differed from group III by $100d=6.66-7.57$ (Shinwari *et al.*, 1994c).

Discussion

The results support the idea of Shinwari *et al.*, (1994c) that *Polygonatae sensu lato* is not a monophyletic group. Three distinct groups can easily be recognized. Group I with genera *Smilacina*, *Maianthemum* and *Disporopsis*; group II with genera *Uvularia* and *Disporum* and group III with genera *Clintonia* and *Prosartes*. The data supports the treatment of these genera in tribe and family by Dahlgren *et al.*, (1985). Hence the RFLP studies turned out to be concordant to morphological data. All these groups can easily be distinguished morphologically from each other.

The present findings suggest that the transfer of *Smilacina* species to *Maianthemum* by LaFrankie (1986) was perhaps reasonable, because the RFLP patterns of all *Smilacina* species were very similar to *Maianthemum* and differ only in ($100d=0.04-0.15$). This distance is similar to the other intrageneric distances. For example, the distance between *Disporum sessile* and *D. taipingense* is 0.34, and 0.03-0.08 among *Prosartes* species.

The results also supports Shinwari *et al.*, (1994b) recognition of *Prosartes* as an independent genus because the DNA pattern of *Disporum* are totally different from *Prosartes* in almost all cases. The DNA of *Disporum* were more similar to *Uvularia* than any other genus.

The present report support the lumping of *Smilacina* in *Maianthemum* as suggested by LaFrankie (1986), because the DNA's of both the genera were very similar in almost all the cases. *rbcL* gene of both the genera is also very similar and differ only in 11 base pairs ($100d=0.82$; Shinwari *et al.*, 1994c). The distance between these two genera was 0.04-0.15. The results also indicate and support Fukuhara & Shinwari's (1994) views that *Uvularia* is closely related to *Disporum* while *Clintonia* is closer to the genus *Prosartes*.

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