Pak.J.Bot., 27(2): 361-369, 1995.

CONGRUENCE BETWEEN MORPHOLOGICAL AND MOLECULAR PHYLOGENETIES IN *PROSARTES* (LILIACEAE)

ZABTA KHAN SHINWARI

Department of Botany,
Faculty of Science, Kyoto University, Sakyo-ku,
Kyoto 606-01, Japan.

Abstract

cp DNA variations were examined in 5 genera of the tribe Polygonatae, for 60 probe-enzyme combinations, to study *Disporum-Prosartes* generic separation problem. The shared fragments were counted, index of similarity and distance matrix was calculated. The distance within the genus *Prosartes* was 0.033, while it differed from *Disporum* in 0.34-0.342. The difference of *Prosartes* from *Streptopus* was 0.217, while between *Disporum* and *Uvularia* was 0.164-0.171. The data was examined using Neighbour Joining (NJ) and Fitch-Margoliash methods. The divergence between *Disporum* and *Prosartes* was greater than that between *Disporum* and *Uvularia* or *Prosartes* and *Streptopus*. Within the genus *Prosartes*, *P. maculata* was different from the rest of the *Prosartes* species.

*rbc*L gene sequence data revealed that north American *Prosartes* and Asian *Disporum* species differed by 90-96 substitution (100d = 6.92-7.41). The base substitution between *Disporum* and *Prosartes* is much greater than that between *Prosartes* and *Streptopus* (14-16, 100d = 1.03-1.18) or that between *Disporum* and *Uvularia* (23-26, 100d = 1.70-2.01).

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

Introduction

Genus *Disporum* comprises about 23 species perennial herbaceous plants characterized by drooping terminal inflorescence. The taxonomy of the genus *Disporum sensu lato* is quite controversial.

The genus *Disporum* was first described by D. Don (1825) and *Prosartes* as an independent genus in 1841. The distribution of the genus is isolated disjunctively into two remote regions; one (*Disporum sensu stricto*) in eastern Asia southward to Ceylon and the eastern Himalayas, and the other (*Prosartes*) in North America. Bentham & Hooker (1883) transferred the then species of *Prosartes* to the genus *Disporum* on the basis of similarity in the habit and some floral characters of the taxa. Lumping of these 2 genera was criticized many times (Jones, 1951; Therman, 1956; Cave, 1970; Sen, 1975; Takhtajan, 1987; Hong & Zhu, 1990; Tamura *et al.*, 1992). Jones (1951) created a section *Prosartes* and kept all North American species of the genus under it.

Morphologically and karyologically *Prosartes* differs from *Disporum* in that, i) anthers are innate in *Prosartes* while adnate in *Disporum*, ii) style entire in *Prosartes*

*Present address: National Herbarium, National Agricultural Research Centre, Park Road, Islamabad, Pakistan.

Table 1. Sources of plant materials.

Species	Localities	Collector (s)	*Accession no.
Disporum D. Don			
D. sessile D. Don	Japan: Kyoto Pref.: Ohmiya-cho, Mt. Takano	Z.K. Shinwari	1
D. taipingense Tamura et Kawano	Taiwan: Illan Hsien: Mt. Tai-Ping	M.N. Tamura	2
Prosartes D. Don			
P. hookeri Torrey	USA: Pennsylvania: Somerset Co., Powder mill	S.Kawano et al.	3
P. trachycarpa Watson	USA: Pennsylvania: Somerset Co., Powder mill	S.Kawano et al.	4
P. smithii (Hooker) Shinwari et al.	USA: California: Patrick Creek	S. Kawano et al.	5
P. maculata (Buckley) Gray	USA: Ohio: Scioto Co., Shewnee st. Forests	S. Kawano et al.	6
P. lanuginosa (Mich.) Don	USA: Ohio: Scioto Co., Lump Black Run	S. Kawano et al.	7
Uvularia grandiflora Linn.	USA: Wisconsin: Marathen Co. Forest	S.Kawano et al.	8
U. sessilifolia Linn.	USA: Pennsylvania: Somerset Co., Powder mill	S. Kawano et al.	9
Tricyrtis affinis Makino	Japan: Kyoto Pref.: Ohmiya-cho, Mt. Takano	Z.K. Shinwari	10
Streptopus lanceolata (Aiton) Reveal	USA: Wisconsin: Marathen Co. Forest	S. Kawano et al.	11

*These numbers are used in Fig.3.

while deeply divided in *Disporum*, iii) the seeds are pendulous in *Prosartes* while ascending in *Disporum*, iv) *Prosartes* has straw-coloured to reddish berries while *Disporum* has dark blue to black berries, v) chromosomes are smaller in *Prosartes* than *Disporum*, and vi) *Prosartes* has prochromosome type (i.e. chromosomes with distinct characters), while *Disporum* possesses a typical homogeneously diffused type of interphase chromosomes.

Takhtajan (1987) reported that chromosomes of Asiatic *Disporum* are similar to *Uvularia* than to *Prosartes*. Conover (1983, 1991) reported that leaf venation and stomatal guard cell pattern of *Prosartes* were quite distinct from those of *Disporum*. Fakuhara & Shinwari (1994) mentioned that the seed coat anatomy of *Disporum* is similar to *Uvularia* and *Prosartes* has distinct type of seed coat structures.

Based on observations of Jones (1951), Therman (1956), Sen (1975), Takhtajan (1987), Hong & Zhu (1990), Tamura *et al.*, (1992) the section *Prosartes* was recognized as an independent genus by Utech *et al.*, (1994) and Shinwari *et al.*, (1994a).

In recent years there has been growing interest in the use of molecular systematics as a tool for solving controversial phylogenetic problems (Ogihara & Tsunewaki, 1982; Shinwari *et al.*, 1994a,b,c). Palmer (1985) and Crawford (1990), have provided a concise and lucid discussion of the potential value and limitations of RFLPs for systematic studies. Such studies have contributed to a better understanding of a host of phylogenetic problems, including the identification of crop plant origins from wild species, identification of maternal and paternal ancestry of a number of hybrid and polyploid species, detection of unexpected cases of introgression, and identification of the progenitor genus of a putatively monotypic, morphologically isolated genus (Palmer 1987; Palmer *et al.*, 1988; Crawford, 1990).

In our first effort (Shinwari *et al.*, 1994a) *rbc*L gene sequence data was obtained for 4 species of *Disporum sensu lato*. To further check the idea of restoration of the genus, seed coat anatomy of a number of closely related taxa was studied (Fukuhara & Shinwari, 1994). In the present study more information on the subject is provided through restriction fragments length polymorphism (RFLP) of two species of *Disporum sensu stricto*, all five species of genus *Prosartes*, two species of genus *Uvularia*, one species each of genus *Streptopus* and *Tricyrtis*.

Materials and Methods

Plant Materials: Eleven accessions of five different genera of tribe *Polygonatae sensu lato* were selected to represent 11 species (Table 1). Voucher specimens have been deposited in the Herbarium of the 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 prewarmed extraction buffer (100mM Tris-HCl, pH 8.0; 50mM EDTA, pH 8.0; 50mM NaCl; 1.25% SDS; 10mM B-merchaptoethanol) was added. After a

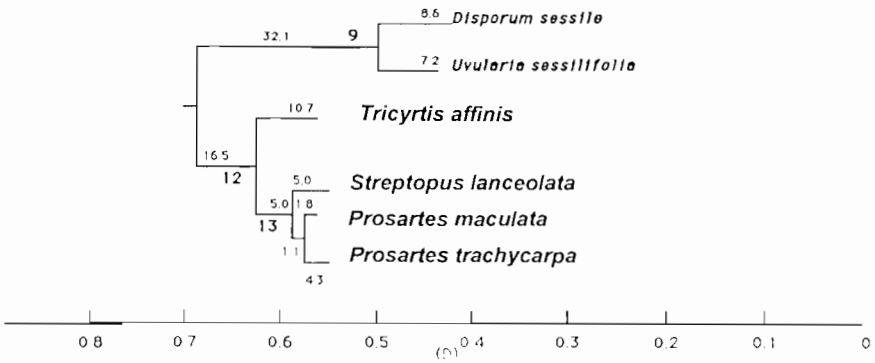

Fig.1. A Neighbor - Joining method tree among 4 genera and 6 species of Liliaceae - Polygonatae, reflecting relationship between Asiatic *Disporum* and American *Prosartes*.

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 600μl-TE, 30μg 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.

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* (Terauchi *et al.*, 1989). The labeling of probes and detection of hybridization were done with a non-radioactive system using DIG-dUTP kit (Boehringer Mannheim).

Phylogenetic trees: A Neighbour Joining (NJ) dendrogram (Fig. 1) Saitou & Nei (1987) and Fitch Margoliash tree (Fig.2) were constructed showing restriction fragment pattern similarity among these 11 taxa. The NJ method is a distance matrix method producing an unrooted tree without the assumption of a clock. NJ method sequentially identifies neighbour pairs that minimize the total length of the tree. Fitch & Margoliash (1967) method proceeds by inserting "missing" OTU's as common ancestors of later OTU's and fits branch lengths to groups of three OTU's at a time.

Results

RFLP analysis: Hybridization of the five cpDNA probes to southern blot with digest of 11 DNA samples for the 12 enzymes were examined. We failed to detect small fragments (< 1kb) predicted to occur because of a restriction site loss/gain. Failure to detect these fragments most likely results from insufficient sensitivity of the southern

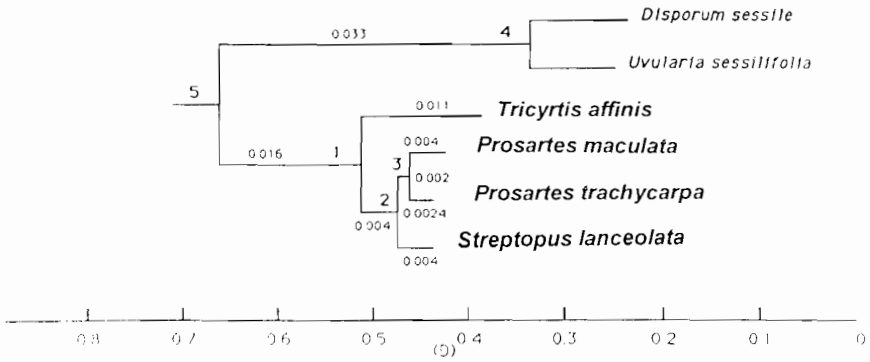

Fig.2. A Fitch Margollash tree among 4 genera and 6 species of Liliaceae - Polygonatae, reflecting relationship between Asiatic *Disporum* and American *Prosartes*.

hybridization to detect small fragments (Fig.3) and that *Disporum* is closer to *Uvularia* than to *Prosartes*, while *Prosartes* is nearer to *Streptopus* than to *Disporum*.

The result obtained by counting shared fragments and calculating the index of similarity and distance matrix shows that the distance within the genus *Prosartes* was 0.033, while it differed from *Disporum* in 0.34-0.342 (Table 2). The difference of *Prosartes* from *Streptopus* was 0.217, while between *Disporum* and *Uvularia* as 0.164-0.171. The data was examined using NJ and Fitch-Margoliash methods. The divergence between *Disporum* and *Prosartes* was greater than that between *Disporum* and *Uvularia* or *Prosartes* and *Streptopus*. Within the genus *Prosartes, P. maculata* was different from the rest of the *Prosartes* species.

*rbc*L gene sequence data revealed that North American *Prosartes* and Asian *Disporum* species differed by 90-96 substitution (100d= 6.92-7.41). The base substitution between *Disporum* and *Prosartes* is much greater than that between *Prosartes* and *Streptopus* (14-16, 100d=1.03-1.18) or that between *Disporum* and *Uvularia* (23-26, 100d=1.70-2.01).

Discussion

The higher order taxonomy as well as intrageneric classification and taxonomic status of *Disporum sensu lato* is currently in a flux. *Uvularia* and *Streptopus* are the genera most commonly associated with *Disporum sensu lato*. Numerous morphological reasons against lumping of *Disporum* and *Prosartes*. (Don, 1841; Hooker, 1883; Takhtajan, 1987; Gleason & Cronquist, 1991) and karyological (Jones, 1951; Therman, 1956; Sen, 1975. Hong & Zhu, 1990. Tamura *et al.*, 1992) were given. The recent studies on micro-morphological leaf characters (Conover 1983, 1991) and seed coat anatomy (Fakuhara & Shinwari, 1994) also recommend generic status for *Prosartes*.

Based on all these observations Utech *et al.*, (1994) and Shinwari *et al.*, (1994) justified the formal recognition of genus *Prosartes*. The results of the present study revealed greater divergence between *Disporium* and *Prosartes* than between other genera of the tribe. Therman (1956) was of the view that the center of diversification of

Table 2. Distance matrix of four genera of Polygonatae.

Genus Name	*Disporum*	*P. maculata*	*Prosartes**	*U. grandiflora*	*U. sessilifolia*	*Streptopus*
Disporum	0.000					
Prosartes maculata	0.340	0.000				
*Prosartes**	0.342	0.033	0.000			
Uvularia grandiflora	0.164	0.500	0.501	0.000		
U. sessilifolia	0.171	0.508	0.514	0.042	0.000	
Streptopus	0.550	0.217	0.214	0.574	0.588	0.000

*Prosartes (except *P. maculata*)

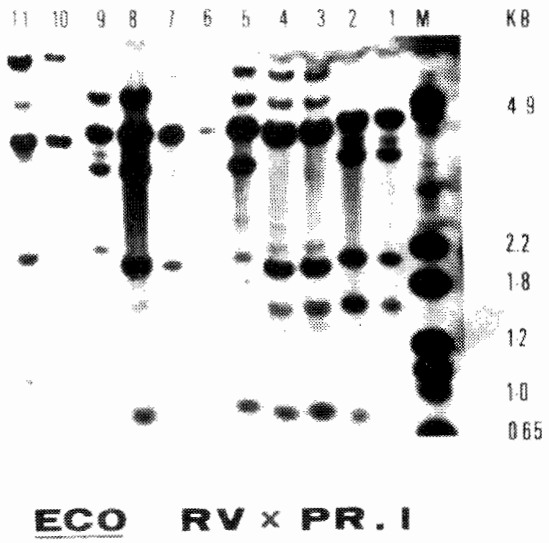

Fig. 3. RFLP patterns. (For accession numbers 1-11 see Table 1).

the whole tribe *Polygonatae* probably lies in Eastern Asia. The size of chromosomes of *Disporum* is much bigger than that of *Prosartes* and *Streptopus*. Therefore, based on all this information we can conclude that *Disporum* in the tribe is a primitive genus and the rest of the genera are rather advanced.

rbcL gene sequence data revealed that North American *Prosartes* and Asian *Disporum* species differed by 90-96 substitution (100d= 6.92-7.41). The base substitution between *Disporum* and *Prosartes* is much greater than that between *Prosartes* and *Streptopus* (14-16, 100d=1.03-1.18) or that between *Disporum* and *Uvularia* (23-26, 100d=1.70-2.01).

The divergence between *Disporum* and *Prosartes* was greater than that between *Disporum* and *Uvularia* or *Prosartes* and *Streptopus*. Within the genus *Prosartes*, *P. maculata* was different from the rest of the *Prosartes* species. Molecular data turned out to be congruent with morphological data in supporting the restoration of the genus *Prosartes*.

Acknowledgement

I wish to express my sincere thanks to Prof. Shoichi Kawano and Dr. Ryohei Terauchi, who helped me in different respects to complete the present work. The present study was financially supported by Grant-in-Aid No. 01041055 from the Monbusho International Scientific Research Program, Japanese Government, to Professor Shoichi Kawano, for which we are grateful. I thank Public Services Division of Pakistan Museum of Natural History, for their help to complete this project. Thanks to all the collectors of the plants which I analyzed in the present work.

References

Bentham, G. and J. D. Hooker. 1883. *Genera Plantarum*. L. Reeve and Co., London. 3: 748-837.

Cave, M.S. 1970, *Chromosomes of the California Liliaceae*. Univ. Calif. Pub.

Conover, M.H. 1983. The vegetative morphology of the reticulate veined Liliiflorae. *Telopea*, 2: 401-412.

Conover, M.H. 1991. Epidermal patterns of the reticulate-veined Liliiflorae and their parallel allies. *Bot. J. Linn. Soc.*, 107: 295-312.

Crawford, D.J. 1990. *Plant Molecular Systematics: Macromolecular Approaches*. John Wiley & Sons Publ. New York, USA.

Don, D. 1825. *Prodromus Florae Nepalensis*. J. Gale, London.

Don, D. 1841. A monograph of *Streptopus*, with the description of a new genus first separated from it. *Trans. Linn. Soc.*, 18: 525-534.

Fakuhara, T. and Z. K. Shinwari. 1994. Seed-coat anatomy of the Northern Hemispheric Uvulariaceae (Liliales): Systematic implications. *Acta Phytotax. Geobot*, 45: 1-14.

Fitch, W. and M. Margoliash. 1967. Construction of Phylogenetic trees. *Science*, 155: 279-284.

Gleason, H. A. and A. Cronquist. 1991. *Manual of vascular plants of northeastern United States and adjacent Canada*. 2nd ed. New York Botanical Garden, Bronx.

Hong, D. and X. Zhu. 1990. Report on karyotypes of 6 species in 4 genera of Polygonatae from China. *Acta Phytotax. Sinica*, 28: 185-198.

Jones, Q. 1951. A cytotaxonomic study of the genus *Disporum* in North America. *Contr. Gray. Herb.*, 173: 1-40.

Ogihara, Y. and K. Tsunewaki. 1982. Molecular basis of genetic diversity among cytoplasm of *Triticum* and *Aegilops* species 1: Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. *Jpn.J. Genet.*, 57: 371-396.

Palmer, J.D. 1985. Comparative organization of chloroplast genomes. *Ann. Rev. Genet.*, 19: 325-354.

Palmer. 1987. Chloroplast DNA evolution and Biosystematic uses of chloroplast DNA variation. *Am. Nat.*, 130: 6-29.

Palmer., J.D., R.K. Jansen, H. J. Michael, M. W. Chase and J. R. Manhart. 1988. Chloroplast DNA variation and plant phylogeny. *Ann. Missouri Bot. Gard.*, 75: 721-726.

Saitou, N. and M. Nei. 1987. The neighbour joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4:406-425.

Sen, S.1975. Cytotaxonomy of Liliales. *Feddes Repert*, 86: 255-305.

Shinwari, Z.K., R. Terauchi, F.H. Utech and S. Kawano. 1994a. Recognition of the New World *Disporum* section *Prosartes* as *Prosartes* (Liliaceae) based on the sequence data of rbcL gene. *Taxon*, 43: 353-366.

Shinwari, Z.K., R. Teranchi and S. Kawano. 1994b. RFLP analysis of cpDNA in several Asiatic *Disporum* species. *Pl. Species Biology.*, 9:11-18.

Shinwari, Z.K., R. Terauchi and S. Kawano. 1994c. Phylogenetic relationships among genera in the *Liliaceae-Asparagoideae-Polygonatae sensu lato* inferred from *rbcL* gene sequence data. *Pl. Syst. Evol.*, 192: 263-277.

Tai, T.H. and S.D. Tanksley. 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Biol. Report*, 8: 297-303.

Takhtajan, A. 1987. *Systema Magnoliophytorum*. 287-309. Leninopoli Officina Editoria "NAUKA" Secto Leninopolitana MCML XXXVII.

Tamura, M.N., F.H. Utech and S. Kawano. 1992. Biosystamatic studies in *Disporum* (Liliaceae-Polygonatae). IV. Karyotype analysis of some Asiatic and North American taxa with special reference to their systematic status. *Pl. Species Biol.*, 7: 103-120.

Terauchi, R., T. Terachi and K. Tsunewaki. 1989. Physical map of chloroplast DNA of aerial yam, *Dioscorea bulbifera* L. *Theor. Appl. Gent.*, 78: 1-10.

Therman, E. 1956. Cytotaxonomy of tribe *Polygonatae*. *Am. J. Bot.*, 43: 134-142.

Utech, F.H., Z.K. Shinwari and S. Kawano. 1994. Biosystematic studies in *Disporum* (Liliaceae) VI. Recognition of the North American section *Prosartes* as an autonomous genus. *Mem. Fac. Sci. Kyoto Univ.* (Ser. Biol.), 16: 1-41.

(Received for Publication 31 August, 1995)