Published in Plant Biosystems 134, issue 3, 385-392, 2000
which should be used for any reference to this work

Phylogenetic relationships and character evolution in *Primula* L.: the usefulness of ITS sequence data

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ABSTRACT - The main goals of this research were to reconstruct the infrageneric phylogeny of the genus *Primula* based on both nuclear and chloroplast DNA sequences, and to use the resulting phylogenies to elucidate the evolution of breeding systems, morphological characters, chromosome number, and biogeographic distribution in the genus. In this paper, the results of a pilot study based on the nuclear ribosomal Internal Transcribed Spacer (ITS) region are described. ITS sequences from 21 taxa produced a number of variable characters sufficient to resolve relationships among sections. The resulting phylogeny confirmed the monophyly of sections *Auricula* and *Aleuritia*. Sections *Armerina*, *Proliferae*, *Crystallophlomis*, *Parryi*, and *Auricula*, with a base chromosome number of x = 11, and sect. *Aleuritia*, with a base chromosome number of x = 9, formed two well supported clades. The ITS topology also suggested that leaves with revolute vernation, previously believed to be a derived state, might represent the ancestral condition in *Primula*, with later reversals to the involute condition. Finally, this initial ITS tree provides preliminary support to the proposed role of the widespread, diploid and heterostylous *P. mistassinica* as having given origin to the polyploid, homostylous *P. incana* and *P. laurentiana*.

KEY WORDS - breeding systems, heterostyly, homostyly, ITS phylogeny, polyploidy, *Primula*

According to the most recent and comprehensive monographic treatment, the genus *Primula L.* (*Primulaceae*) includes over 400 species subdivided into 6 subgenera and 37 sections (RICHARDS, 1993). About half of the species occur in the Eastern Sinohimalaya, the primary centre of diversity for the genus. Primulas include mostly perennial herbs with a few annuals (HU & KELSO, 1996). Leaves are simple, and arranged in a basal rosette (ANCHISI *et al.*, 1987). Flowers are clustered in umbellate, racemose, subcapitate, or spicate inflorescences. Individual flowers are typically sympetalous, with a pentamerous corolla and 2-cleft corolla lobes. Heterostyly is common in *Primula*, and was the basis for the first careful study of this floral phenomenon by DARWIN (1877). Stamens are adnate to the corolla tube and have very short filaments. The superior ovary ripens into a capsule that dehisces by valves, releasing numerous small seeds (HU & KELSO, 1996). Most species of *Primula* have

either articulated hairs or glands on at least some of their vegetative parts (KELSO, 1991b). Glandular development is most pronounced in species that show abundant farina, a powdery exudate composed primarily of flavones (HARBOURNE, 1968). Heterostyly (distyly) and homostyly, which occur respectively in 90% and 10% of the species, are usually reliable taxonomic characters at the species level (KELSO, 1991b), although heterostylous and homostylous species may be found together in the same sections of the genus (see BARRETT, 1992, for a review of heterostyly in angiosperms). The proposed base chromosome number is x = 11, with variation between x = 8 and x = 12. Chromosome number is remarkably stable within groups and it has been used as an important character in sectional classification (RICHARDS, 1993).

A number of recent monographic studies of *Primula* (WENDELBO, 1961; SCHWARZ, 1968; SMITH *et al.*, 1977; FENDERSON, 1986; ANCHISI *et al.*, 1987; HALDA, 1992; RICHARDS, 1993) testify to the widespread interest in the genus and provide us with a wealth of morphological, biogeographic, ecological, and cytological information that can be profitably analysed in a phylogenetic context. Despite the wealth of published information on the morphology and reproductive biology of *Primula* (WEDDERBURN & RICHARDS, 1990) no explicit, rigorous phylogenetic analysis derived from either morphology or molecules has been published to date. The most recent monographic treatment (RICHARDS, 1993) suggested hypotheses of intersectional relationships, but this study interpreted polarity based on a subjective assessment of the primitive and derived condition for each character. A cladistic morphological analysis of the order *Primulales* (ANDERBERG & STAHL, 1994), that included two species of *Primula*, placed *Dionysia* in the same clade with the two Primulas, suggesting that *Primula* may not form a monophyletic unit.

Our study will ultimately provide the first comprehensive and rigorous infrageneric phylogenetic analysis of *Primula* derived from both nuclear and chloroplast DNA sequences, while continued studies by Anderberg (personal communication) will examine the circumscription of the genus within the family. This paper presents preliminary results based on DNA sequences from the nuclear ribosomal Internal Transcribed Spacer (ITS) region that show its usefulness for resolving the infrageneric phylogeny of *Primula*. These results, derived from limited sampling within the genus (19 species), allowed us to evaluate the phylogenetic validity of existing sectional classifications and to propose likely scenarios for the evolution of chromosome numbers, leaf vernation, and breeding systems.

MATERIALS AND METHODS

DNA was extracted from frozen or silica-gel dried leaves from single individuals representing 19 species of *Primula*, classified in six different sections by RICHARDS (1993), and two species of *Douglasia*, which were used for global outgroup comparison (see Table 1). Two extraction methods were used on different species: a modified CTAB extraction procedure (DOYLE & DOYLE, 1987) or the DNeasy Plant Mini Kit (QiaGen). The nuclear DNA fragments to be used as sequencing templates were symmetrically amplified with the polymerase chain reaction in a Perkin Elmer 2400 Thermal Cycler. Before sequencing, the PCR products were run on a 1% agarose gel, sliced out if necessary and purified with the QIAquick Gel Extraction Kit (QiaGen). Sequencing reactions were performed with dye-terminator cycle sequencing kits (Applied Biosystems) in a Perkin Elmer 2400 Thermal Cycler, purified with a 90% ethanol precipitation, and sequenced on an Applied Biosystems 373A automated DNA sequencer. The ITS region, including ITS 1, the 5.8 S rDNA, and ITS 2, was amplified with the primer pair C26A and LeuF and sequenced with the internal primers 1F, 2R, 3F, and 4R (BALDWIN *et al.*, 1995). Partial sequences were assembled using Sequence Navigator (Applied Biosystems) and easily aligned by eye, as few indels were present (CONTI *et al.*, 1999). The present paper presents the results of a pilot study, therefore we deemed it premature to score indels as additional characters, because the scoring of such characters will change as the ITS data set grows and the alignment changes.

The maximum parsimony (MP) optimality criterion, as implemented in PAUP 4.0b2a (SWOFFORD, 1999), was used to infer phylogenies. Most parsimonious trees were built using the branch and bound search with the addition method set at furthest. All characters were unordered and equally weighted and gaps were treated as missing. Tree statistics were calculated and statistical support for each branch of the most parsimonious trees was assessed with bootstrap analysis conducted on the original data matrix by generating 100 pseudoreplicates and by using the branch and bound search. *Douglasia alaskana* and *D. beringensis* were used for global outgroup comparison and were allowed to be paraphyletic with the 19 species of *Primula* (MADDISON *et al.*, 1984).

RESULTS

The sequenced ITS and 5.8 S regions varied in length from 757 nucleotides (NTPs) in *Douglasia beringensis*,

TABLE 1
List of specimens used in this study. Classification according to RICHARDS (1993)

Species	Subgenus	Section	Subsection	Collection data	GenBank number
P. tschuktschorum Kjellman	*Aleuritia* (Duby)Wendelbo	*Crystallophlomis* (Rupr.) Federov	*Crystallophlomis*	D. Murray 12,280 #1, 2 July 97. Seward Pen, AK	AF260767
P. eximia Greene	*Aleuritia* (Duby)Wendelbo	*Crystallophlomis* (Rupr.) Federov	*Crystallophlomis*	C. Parker, June 97. Denali Nat. Park, AK	AF260768
P. nutans Georgi	*Aleuritia* (Duby)Wendelbo	*Armerina* Lindley	NA	T. Kelso, July 92. Nome, AK	AF260757
P. secundiflora Franchet	*Aleuritia* (Duby)Wendelbo	*Proliferae* Pax	NA	E. Conti, 3 Aug 95. Tromsoe Bot. Gard. 92-1051	AF260759
P. angustifolia Torrey	*Auriculastrum* Schott	*Parryi* W.W. Smith ex Wendelbo	NA	T. Kelso, July 92. Penn Mountain, CO	AF260754
P. glutinosa Wulfen in Jacq	*Auriculastrum* Schott	*Auricula* Duby	*Cyanopsis* Schott	E. Conti, July 95. Tromsoe Bot. Gard. 95-598	AF260755
P. minima L.	*Auriculastrum* Schott	*Auricula* Duby	*Chamaecallis* Schott	E. Conti, July 95. Tromsoe Bot. Gard. 92-1156	AF260756
P. spectabilis Tratt	*Auriculastrum* Schott	*Auricula* Duby	*Arthritica* Schott.	E. Conti, 3 Aug. 95. Tromsoe Bot. Gard. 93-60	AF260760
P. clusiana Tausch	*Auriculastrum* Schott	*Auricula* Duby	*Arthritica* Schott.	E. Conti, 3 Aug. 95. Tromsoe Bot. Gard. 95-611	AF260761
P. palinuri Petagna	*Auriculastrum* Schott	*Auricula* Duby	*Auricula*	A. Anderberg, SU-S-00.96.2	AF260758
P. modesta Biss. & Moore	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	L. Hufford, April 95. Kew Gardens 1981-1718	AF260762
P. laurentiana Fernald	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	T. Kelso, 1998. Horticultural source	AF260763
P. borealis Duby	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	C. Parker 7537, July 97. Seward Pen., AK	AF260764
P. incana M.E. Jones	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	C. Parker, 18 June 97. Fairbanks, AK	AF260771
P. mistassinica Michx	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	C. Hellquist, 1996. Lake Michigan	AF260770
P. stricta Hornemann	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	E. Conti, 3 Aug. 95. Tromsoe Bot. Gard 92-4055	AF260766
P. farinosa L.	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	L. Hufford, April 95. Kew 1992-629	AF260772
P. frondosa Janka	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	T. Kelso, 1998. Horticultural source	AF260765
P. scandinavica Bruun	*Aleuritia* (Duby)Wendelbo	*Aleuritia* Duby	NA	E. Conti, 3 Aug 95. Tromsoe Bot. Gard.	AF260769
Douglasia alaskana Coville & Standley ex Hultén	NA	NA	NA	C. Parker 6960, 23 June 97. Thorofore Pass, AK	AF260774
Douglasia beringensis S. Kelso, Jurtsev, & d.F. Murray	NA	NA	NA	D. Murray, 4 July 97. Seward Pen., AK	AF260773

NA: not applicable.

immediately followed by *D. alaskana* (758 NTPs), to 793 NTPs in *Primula mistassinica*. The shortest ITS regions in *Primula* belonged to *P. tschuktschorum* and *P. clusiana* and were 774 NTPs long. The lowest nucleotide differences (D) were found within sections, specifically between the following pairs of ITS sequences: *P. incana* and *P. laurentiana* (D = 0), *P. incana* and *P. borealis* (D = 0), *P. borealis* and *P. laurentiana* (D = 1), *P. incana* and *P. modesta* (D = 4), *P. scandinavica* and *P. frondosa* (D = 4), *P. incana* and *P. mistassinica* (D = 6) (section *Aleuritia*); *P. eximia* and *P. tschuktschorum* (D = 1) (section *Chrystallophlomis*); and *D. alaskana* and *D. beringensis* (D = 6). Between sections, the lowest nucleotide difference was found between section *Armerina* and section *Crystallophlomis* (*P. nutans* and *P. eximia*: D=9) and the highest between section *Aleuritia* and section *Auricula* (*P. stricta* and *P. glutinosa*: D=108). Total nucleotide differences between *Primula* and *Douglasia* ranged from D = 108 (*D. alaskana* and *P. nutans*) to D = 144 (*D. alaskana* and *P. minima*).

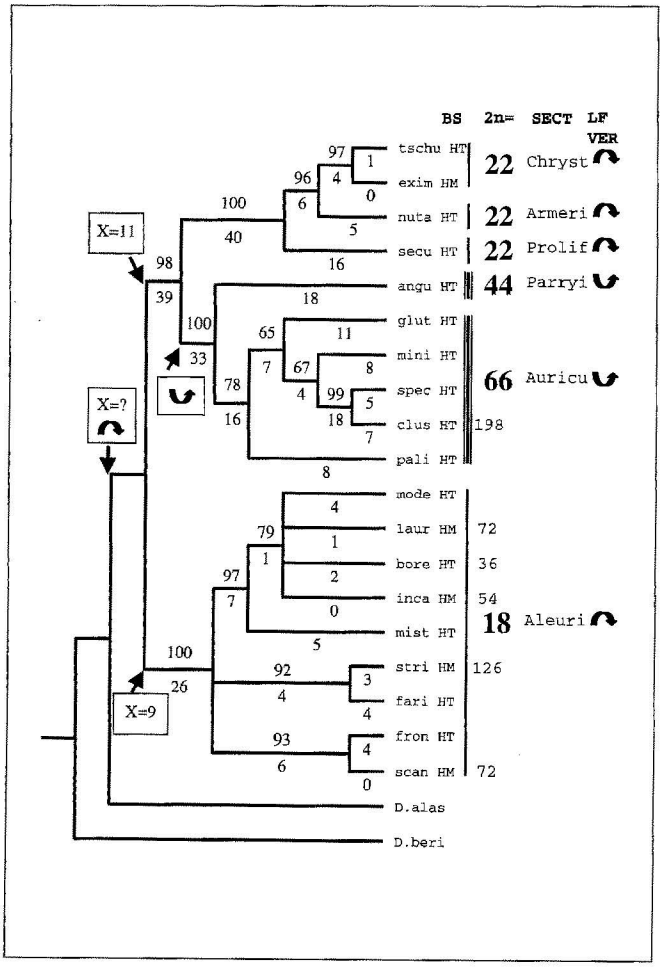

FIGURE 1 - Strict consensus ITS tree. Numbers above the branches indicate bootstrap values, in percent; numbers below the branches indicate branch lengths of one of the ten MP trees. The following characters are reported to the right of the tree: breeding system (BS-HT: heterostyly, HM: homostyly); chromosome number (2n=); and leaf vernation (LF VER: ∪: involute, ∩: revolute). Abbreviations- SECT: sectional affiliation; Chryst: sect. *Crystallophlomis*; Armeri: sect. *Armerina*; Prolif: sect. *Proliferae*; Parryi: sect. *Parryi*; Auricu: sect. *Auricula*; Aleuri: sect. *Aleuritia*; tsch: *Primula tschuktschorum*; exim: *P. eximia*; nuta: *P. nutans*; secu: *P. secundiflora*; angu: *P. angustifolia*; glut: *P. glutinosa*; mini: *P. minima*; spec: *P. spectabilis*; clus: *P. clusiana*; pali: *P. palinuri*; mode: *P. modesta*; laur: *P. laurentiana*; bore: *P. borealis*; inca: *P. incana*; mist: *P. mistassinica*; stri: *P. stricta*; fari: *P. farinosa*; fron: *P. frondosa*; scan: *P. scandinavica*; D. alas: *Douglasia alaskana*; D. beri: *D. beringensis*.
Other symbols- |: subgenus *Aleuritia*; ||: subgenus *Auriculastrum*.

The aligned sequences were 823 NTPs long. Of the aligned nucleotide positions, 34 were gapped, 515 were constant, and 90 were variable but uninformative. The total number of potentially informative characters was 218. The Branch and Bound search found ten most parsimonious (MP) trees that were 417 steps (L) long, if only the informative characters were included, and had a Consistency Index (CI) of 0.777, a Retention Index (RI) of 0.896, and a Rescaled Consistency Index (RC) of 0.697. When all characters were included, the trees were 514 steps long, had a CI of 0.819, and a RC of 0.734.

The strict consensus of the 10 MP trees (see Figure 1) showed a good degree of resolution and the results of the bootstrap analyses provided high statistical support for most of the clades. The ITS tree identified two major clades among the represented species of *Primula*: one clade, with a bootstrap support (BS) of 100%, included all representatives of sect. *Aleuritia* and the other clade (BS = 98%) comprised sequences representing sections *Crystallophlomis*, *Armerina*, *Proliferae*, *Parryi*, and *Auricula*. Within this latter clade, the two subclades that included sections *Crystallophlomis*, *Armerina* and *Proliferae*, and sections *Parryi* and *Auricula*, respectively, also received maximum bootstrap support (BS = 100%).

DISCUSSION

Even though single-gene trees should be viewed with caution as accurate representations of species trees (DOYLE, 1992), ITS trees have proven useful for reconstructing the phylogenetic histories of several plant groups, including other arctic-alpine genera like *Saxifraga* (CONTI *et al.*, 1999). We present these results as the first contribution towards phylogenetic reconstruction in *Primula*. The results of phylogenetic analyses based on ITS sequences from these 19 species showed that this nuclear region provides a number of variable characters (218) sufficient to resolve relationships among the major clades represented in this initial

sampling of the genus. Resolution was lower, however, at the tips of the tree, as indicated by two polytomies within section *Aleuritia,* and by two lower bootstrap values (65% and 67%) within section *Auricula* (see Figure 1).

One of the major goals of our ongoing phylogenetic study of *Primula* is to assess the validity of sectional circumscriptions established on the basis of morphological and cytological characters (FENDERSON, 1986; KELSO, 1987, 1991a, 1991b; RICHARDS, 1993). The study reported here included multiple representatives of three sections: *Aleuritia* (nine out of 28 species, or 32%), *Auricula* (five out of 21 species, or 24%), and *Crystallophlomis* (two out of 31 species, or 7%). The monophyly of these three sections was strongly supported in the ITS tree, with BS values of 100% for sect. *Aleuritia*, 78% for sect. *Auricula*, and 97% for sect. *Crystallophlomis*. Despite the relative lack of fine intrasectional resolution noted above, the ITS tree soundly confirmed (BS = 99%) the sister relationship between *P. spectabilis* and *P. clusiana*, two of the four species ascribed to subsection *Arthritica* Schott of sect. *Auricula* (FENDERSON, 1986). Thus sectional and intrasectional circumscriptions inferred from the ITS tree were congruent with morphological classifications recently proposed by FENDERSON (1986), KELSO (1987, 1991a, 1991b), and RICHARDS (1993).

Our analyses also suggest that the ITS phylogeny of *Primula* is congruent with chromosome number. In this genus the base number varies greatly between sections, ranging from x = 12 to 11, 10, 9, and 8, yet it is remarkably stable within sections (RICHARDS, 1993). The two major clades of the *Primula* ITS tree were supported by high bootstrap values and each was marked by a different chromosome base number: x = 9 for the clade formed by sect. *Aleuritia* and x = 11 for the clade containing sects. *Crystallophlomis*, *Armerina*, *Proliferae*, *Parryi*, and *Auricula* (see Figure 1). The two strongly supported subclades (BS = 100%) in the latter group were also congruent with chromosome number: 2n = 22 was shared by sections *Crystallophlomis*, *Armerina*, and *Proliferae* and a higher ploidy level was shared by sect. *Parryi* (2n = 44) and sect. *Auricula* (2 = 66; see Figure 1). The congruence of the ITS tree with chromosome number lends preliminary support to the taxonomic interpretation of WENDELBO (1961), FENDERSON (1986), and RICHARDS (1993), who dismantled the heterogeneous sect. *Farinosae* as defined by SMITH & FLETCHER (1943). Sect. *Farinosae* contained over 80 species that differed with respect to chromosome base number, which ranged from x = 11 to 10, 9, and 8, leaf morphology, and gland and pollen anatomy. RICHARDS (1993), in particular, subdivided sect. *Farinosae* into six sections, two of which, sect. *Armerina* and sect. *Aleuritia*, were included in this study. The ITS consensus tree strongly supported the inclusion of these two sections in two different clades: sect. *Aleuritia* formed its own clade (BS = 100%) and sect. *Armerina* was included in the second major clade with four other sections (BS = 98%). Within this latter subclade, sect. *Armerina* formed a strong sister group relationship with sect. *Crystallophlomis* (BS = 96%), further rejecting any potential affinity with sect. *Aleuritia*.

The topology of this initial ITS tree, with the basal split between the x = 9 and the x = 11 clades, did not allow us to make inferences about the likely base chromosome number of the ancestral *Primula* (see Figure 1). The monophyly of the clade with base chromosome number x = 11 did not support the hypothesis proposed by RICHARDS (1993) of a paraphyletic *Primula* group with x = 11 from which sections with other chromosome numbers were derived. A more complete sampling of sections of the genus with different chromosome numbers, beyond the current sampling of sections with x = 11 and x = 9, will allow further evaluation of RICHARDS' (1993) hypothesis and could change the conclusions drawn here.

The ITS tree suggests some preliminary conclusions on the evolutionary significance of leaf vernation. Leaf vernation is a useful taxonomic character in the *Primulaceae*: most genera, outside of *Primula*, have invariable leaf vernation, characterised by involute leaves. In *Primula*, however, both revolute and involute leaf vernation can occur. Vernation is remarkably stable within sections, where 89% show the revolute form (RICHARDS, 1993), but a few show the involute form. These few sections have been linked on the basis of involute vernation into the subgenera *Sphondylia* Duby and subgenus *Auriculastrum* Schott. Several authors (WENDELBO, 1961; RICHARDS, 1993) have suggested that involute vernation represents the "primitive" condition for *Primula* based on its common occurrence in the family as a whole. However, the ITS topology suggests that the ancestral *Primula* most likely had revolute leaf vernation, with a later reversal to the involute condition in the common ancestor of sects. *Auricula* and *Parryi* (see Figure 1). The affinity between sections *Auricula* and *Parryi* is further supported by morphology, as the resemblance of glands, pollen, and seeds in these two sections is striking. Future studies that include ITS sequences from section *Cuneifolia* (also characterised by involute vernation) will allow us to clarify whether sections *Parryi*, *Auricula*, and *Cuneifolia* indeed share a common origin. The only other section of *Primula* with involute vernation is sect. *Sphondylia*, which WENDELBO (1961) considered to represent the most primitive group within

Primula. Additional ITS sequences from species of sect. *Sphondylia* will be critical for resolving the issue of whether involute or revolute leaf vernation represents the ancestral condition in the genus.

Finally, the ITS tree presented here, although based on a small sample of the genus, provides initial insights on the evolution of breeding systems and ploidy level in *Primula*. The association between homostyly, polyploidy, and extreme arctic or alpine conditions has been reported for several species of *Primula*. KELSO (1987, 1991b, 1992), elaborating upon STEBBINS' (1950, 1985) secondary contact model, proposed the following sequence of events to explain the evolution of secondary homostyly and polyploidy in *Primula*: 1) Diploid, heterostylous taxa of *Primula* became isolated as a result of habitat fragmentation caused by climate changes, for example Quaternary glacial peaks (HOPKINS, 1982); 2) following glacial retreat, range expansion occurred and allowed some of these diploid taxa to come in contact and hybridise, thus giving rise to allopolyploid taxa; 3) recombination in the heterostyly supergene gave origin to self-fertile, secondary homostylous taxa; 4) these taxa successfully colonised the new habitats opened by glacial retreat. Documented shifts in insect fauna during Quaternary glaciations (MATTHEWS, 1974) possibly created the selective force of reduced pollinator availability to drive the competitive success of homostylous, self-fertile polyploid species as compared to the heterostylous diploids which are obligate outcrossers (BARRETT, 1992). Other studies suggested that similar pollinator limitation provided the selective force driving the evolution of self-fertile races in *Laevenworthia* (*Brassicaceae*) (SOLBRIG & ROLLINS, 1977).

Detailed hypotheses of relationships between diploid, distylous progenitors and polyploid, homostylous derivative species have been proposed especially for sects. *Aleuritia, Armerina*, and *Crystallophlomis* (KELSO, 1987, 1991a, 1991b, 1992). Sect. *Aleuritia*, with its abundance of diploid heterostylous species and polyploid homostylous species, represents a group of primary interest for investigating the proposed correlation between polyploidy, homostyly, and extreme arctic and alpine conditions. RICHARDS (1993) noted that the highest polyploids in sect. *Aleuritia*, *P. stricta* (14x), *P. laurentiana* (8x), *P. scandinavica* (8x) and *P. magellanica* (8x) do not occur south of latitude 60° N or north of latitude 50° S. Parallel patterns occurred in North America and Eurasia, reflecting comparable climatic and geologic histories during the Pleistocene. In Eurasia, the distylous, diploid *P. farinosa*, *P. modesta* and *P. serrata* have been proposed as possible progenitors of the heterostylous, tetraploid *P. borealis*, with amphiberingian distribution, and *P. farinosa* has been proposed as the progenitor of the European polyploids *P. scotica* and *P. scandinavica* (HULTGARD, 1993). In North America, isolates of the diploid *P. mistassinica* likely played a role in the origin of the homostylous hexaploid *P. incana* and octaploid *P. laurentiana*. Two possible progenitor pairs have been proposed for the 14-ploid, amphi-atlantic *P. stricta*: the North American *P. incana* and *P. laurentiana* or the European *P. scotica* and *P. scandinavica* (KNABEN, 1982). Even with limited sampling, the ITS topology allows us to make some preliminary suggestions about the relationships proposed above. First, the ITS tree strongly supports (BS = 97%) a clade that includes the North American *P. mistassinica*, *P. incana*, *P. borealis*, *P. laurentiana*, and the Eurasian *P. modesta*, which occurs primarily in Japan and the Kurile Islands. Within this clade, only one nucleotide difference separates *P. mistassinica* from the hypothetical common ancestor of the subclade including *P. modesta*, *P. laurentiana*, *P. borealis* and *P. incana*. The relationships of *P. mistassinica* supported by the ITS tree lent initial reinforcement to the hypothesis (KELSO, 1991b, 1992) that this widespread, diploid, heterostylous species might have given origin to polyploid, homostylous species like *P. incana* and *P. laurentiana*. Similarly, the inclusion of the Eurasian *P. modesta* in the same polytomous clade with *P. borealis* provides some, although very preliminary, support for the proposed origin of *P. borealis* from *P. modesta*, with which it morphologically intergrades in the Kurile Islands (RICHARDS, 1993).

In summary, ITS sequence data proved useful for resolving the basic phylogenetic structure within *Primula*. Ultimately, more intensive taxon sampling and sequences from other nuclear DNA regions, for example the External Transcribed Spacer region (BALDWIN & MARKOS, 1998; BENA *et al.*, 1998) and from chloroplast DNA regions, for example the *maturase* K gene (SOLTIS *et al.*, 1996; SANG *et al.*, 1997) and the inter-genic spacers (TABERLET *et al.*, 1991) will provide detailed biparental and maternal phylogenies of the genus that will help elucidate questions of hybrid origin for many polyploid, homostylous taxa. As phylogenetic accuracy depends on both number of characters and number of taxa (GRAYBEAL, 1998; BREMER *et al.*, 1999), we believe that a study that includes several DNA regions and nearly exhaustive sampling within different sections, and ultimately within the entire genus, will approach the most likely reconstruction of evolutionary history in *Primula*. This detailed phylogeny will be essential for elucidating the evolution of breeding systems and ploidy level in this fascinating genus.

ACKNOWLEDGEMENTS

The authors wish to thank the Research Corporation and the University of Alaska for partial funding and David Murray, Carolyn Parker, Alan Batten, Carl Roland, and Giorgio Pellegrino for help with the collections. Special thanks go to Peter Raven, John Richards, and Spencer Barrett for providing initial encouragement for this research.

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