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CONSERVATION OF MICROSATELLITES AMONG TROPICAL TREES (LEGUMINOSAE)[1]

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Although microsatellites or simple sequence repeats (SSRs) have become a popular tool in genetic mapping and gene flow studies, their utility is limited due to paucity of information about DNA sequences in plants. We tested the utility of microsatellite markers characterized for the tropical tree *Pithecellobium elegans* as a genetic tool for related species. The results indicate that SSR loci are conserved among closely related species, and SSR primers developed for *P. elegans* could be successfully used as a genetic tool in several species of the tribe Ingeae. This study indicates that there is high potential for the transfer of SSR markers among closely related taxa, circumventing laborious cloning and screening procedures involved in characterizing SSR loci for many species.

Key words: Ingeae; Mimosoideae; phylogeny; *Pithecellobium*; *rbcL*; simple sequence repeat.

In recent years, microsatellites or simple sequence repeats (SSRs) have become a popular tool for genetic mapping (Dietrich et al., 1992; Hearne, Gosh, and Todd, 1992; Weissenbach et al., 1992), and analysis of paternity and gene flow (Amos, Scholtterer, and Tautz, 1993; Chase et al., 1996). This popularity stems from the high allelic diversity found at the SSR loci, the codominance of alleles, and the relatively simple polymerase chain reaction (PCR)-based screening methods. The utility is limited due to the paucity of DNA sequence information for natural populations. Thus costly and laborious cloning and screening procedures limit the number of species that can be studied. In general, SSR markers have been characterized for each species being studied. It is possible that SSR primers developed for one species can be used to detect polymorphism at ''homologous'' loci of a related species. For several groups of animals, the utility of heterologous primers for the identification of SSRs has been investigated (Schlotterer, Amos, and Tausz, 1991; Fitzsimmons, Moritz, and Moore, 1995; Glenn et al., 1996), but there are few such studies with plant groups. Thomas and Scott (1993) and Saghai-Maroof et al. (1994) characterized SSR variability among grapevine cultivars and a worldwide collection of barley, respectively. Wu and Tanksley (1993) have assessed SSR diversity for accessions of rice and showed that the primers were capable of identifying SSRs in conspecific taxa. Kijas, Fowler, and Thomas, (1995) examined several interfertile cultivars and species of the genera *Citrus* and *Poncitrus* and also found that the identification of SSRs from closely related taxa was possible. No study in plant groups has examined the conservation and allelic diversity of SSRs at higher taxonomic scales, nor has any study examined the variability of SSRs within populations of these more distantly related taxa.

Here we examine the extent to which SSR primers developed for one tropical tree species can be used to amplify SSR loci in other species. We used the primers developed for a tropical rain forest tree, *Pithecellobium elegans*, to detect SSR loci in selected species of the same family (Leguminosae). Because the species differ in the degree of relatedness to *P. elegans*, the tests allow us to assess the correlation between primer homology and genetic relatedness based on chloroplast-encoded *rbcL* gene sequence data.

Specifically, we address the following questions: (1) Can the SSR primers developed for one species be used to amplify SSR markers for related species? (2) Is the cross compatibility of SSR primers correlated with genetic relatedness, and at what taxonomic level are these primers potentially useful? (3) Do related species have similar allelic diversity at a given locus? We also present the phylogenetic tree of selected legume species based on *rbcL* sequence data.

MATERIALS AND METHODS

We selected two to six individuals from 13 species in the Leguminosae, 12 species from the subfamily Mimosoideae, and one species from Papilionoideae (Table 1).

DNA was extracted following a protocol modified from Bernatzky and Tanksley (1986). About 200–300 mg of fresh leaves were placed in a plastic bag (Ziplock brand freezer bags) with 2 mL of ice cold extraction buffer (0.35 mol/L sorbitol, 100 mmol/L Tris pH 7.5, 5 mmol/L EDTA, and 0.02 mol/L sodium bisulfite), and crushed with a roller. The extract was transferred to a microcentrifuge tube and placed on ice. Tubes were centrifuged at 6000 rpm for 5 min to pellet nuclei. The supernatant was discarded and the pellet was resuspended in 300 μL of extraction buffer and 300 μL of nuclei lysis buffer (0.2 mol/L Tris [pH 7.5], 0.05 mol/L EDTA, 2 mol/L NaCl, and 2% CTAB [Hexadecyltrimethylammonium bromide]). Sarkosyl (120 μL of 5%) was added and incubated at 65°C for 15–30 min. Chloroform:isoamyl alcohol (24:1, 600 μL) was added and inverted to form an emulsion. Tubes were centrifuged at 7000 rpm for 5 min and the upper phase was transferred to a clean microfuge tube containing 600 μL of isopropanol. The tubes were inverted several times to mix, and then centrifuged at 14 000 rpm for 10 min. The pellet was washed with 70% ethanol, dried, and dissolved in 150 μL of TE (10 mmol/L Tris [pH 7.5], 1 mmol/L EDTA). Five microlitres of the prepared DNA were used for each PCR reaction.

Eight SSR loci were previously characterized for the species *Pithe-*

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TABLE 1. Taxonomy, source (*rbcL* genbank accession number or country from which samples collected), and number of SSR loci amplified (no. SSR) of selected taxa.

Species	Family	Subfamily	Tribe	Source	No. SSR
Pithecellobium elegans	Leguminosae	Mimosoideae	Ingeae	Costa Rica	6
Balizia pedicellaris	Leguminosae	Mimosoideae	Ingeae	Brazil[a]	6
Enterolobium schomburgkii	Leguminosae	Mimosoideae	Ingeae	Brazil[a]	6
Pithecellobium saman	Leguminosae	Mimosoideae	Ingeae	Costa Rica	2
Pithecellobium longifolium	Leguminosae	Mimosoideae	Ingeae	Costa Rica	5
Enterolobium cyclocarpum	Leguminosae	Mimosoideae	Ingeae	Costa Rica	4
Pithecellobium dulce	Leguminosae	Mimosoideae	Ingeae	Costa Rica	6
Albizzia caribaea	Leguminosae	Mimosoideae	Ingeae	Costa Rica	5
Parkia pendula	Leguminosae	Mimosoideae	Parkieae	Brazil[a]	0
Pentaclethra macroloba	Leguminosae	Mimosoideae	Parkieae	Costa Rica	0
Acacia collincii	Leguminosae	Mimosoideae	Acaciae	Costa Rica	0
Dinizia excelsa	Leguminosae	Mimosoideae	Mimoseae	Brazil[a]	0
Cercis canadensis	Leguminosae	Caesalpinioideae	Cercideae	Z70163	NA
Cercis siliquastrum	Leguminosae	Caesalpinioideae	Cercideae	Z70164	NA
Gliricidia sapium	Leguminosae	Papilionoideae	Robinieae	Costa Rica	0
Medicago sativa	Leguminosae	Papilionoideae	Trifolieae	Z70173	NA
Pisum sativum	Leguminosae	Papilionoideae	Vicieae	X03853	NA
Polygala cruciata	Polygalaceae			L01945	NA
Securidaca diversifolia	Polygalaceae			L01955	NA
Cupaniopsis anacardioides	Sapindaceae			L13182	NA
Gossypium hirsutum	Malvaceae			X15886	NA

[a] Samples collected by Chris Dick, Harvard University Herbaria, Cambridge, MA.

cellobium elegans (Chase, Kesseli, and Bawa, 1996; Chase et al., 1996). Of these, six pairs of primers (Table 2) were used to amplify DNA of related species in this study. After an initial denaturation step at 94°C, five cycles were performed with 94°C denaturation, 55°C annealing, and 72°C extension temperatures, 1 min at each step, followed by 30 cycles at the same tempertures, but with 30 s at each step. Each amplification was performed in a total volume of 15 μL, which consisted of 0.2 mmol/L dNTP, 2.0 mmol/L MgCl2, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 pmol of reverse primer, 2 pmol of forward primer, 0.5 pmol (0.5uCi) of P33 endlabelled forward primer, and 1.5 μL Taq DNA polymerase. Primer end labelling was done with polynucleotide kinase from Epicentre Technology . The labelling mix consisted of 3μL of 10X PNK buffer, 12.5 μL of H_2O, 1 μL of T4 polynucleotide kinase, 1.5 μL of Gamma P33 labelled ATP, and 12 μL of oligonucleotide primer (30 pmole). The reaction mix was incubated at 37°C for 30 min and then the enzyme was inactivated at 70°C for 5 min. After thermal cycling, 10 μL of stop solution (Xylene cyanol, Bromophenol blue in deionized formamide) was added. The above amplifications were also repeated at a 45°C anealing temperature.

Amplified products were electrophoresed on 6% denaturing gel with 6 mol/L urea and TBE. Electrophoresis was performed on a S2 electrophoretic apparatus (Owl Scientific Inc., Woburn, Massachusetts) for ~4 h at settings of 1750 V, 55 W, or until xylene cyanol marker migrated ~35–40 cm from the origin.

To reconstruct the phylogeny of the taxa being studied, we selected nucleotide sequences of the chloroplast-encoded *rbcL* gene. The *rbcL* gene was amplified using oligonucleotide primers G1F and G1460R, homologous to the *Gossypium rbcL* gene (Dayanandan, 1995). Amplification reactions contained 200 μmol/L each of dATP, dCTP, dGTP, dTTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), 0.1% Triton X-100, 10 pmol of each primer, 2 units of Taq DNA Polymerase, and 1.5 mmol/L MgCl2 in a total volume of 100 μL. Thermal cycling was performed in a PTC100 thermal cycler (MJ Research, Watertown, Massachusetts) at 94°C for 90 s, 40°C for 60 s, and 72°C for 120 s for 40 cycles. Amplified DNA was purified using Wizard PCR purification columns (Promega Corporation, Madison, Wisconsin) and directly sequenced by Sanger dideoxy chain termination method with thermal cycling using ABI prism 377 automated DNA sequencer (Perkin-Elmer, Applied Biosystems, Foster City, California). Thermal cycling was performed at 30 s at 50°C, 60 s at 70°C, and 90 s at 94°C for 30 cycles. Ten internal primers (Dayanandan, 1995), modified from primers designed by Gerard Zurawski (DNAX Institute, Palo Alto, California) were used for sequencing both strands of the *rbcL* gene. Published *rbcL* sequences of *Polygala cruciata* and *Securidaca diversifolia* of Polyga-

TABLE 2. Oligonucleotide primer sequences, repeat pattern, and number of alleles in *P. elegans* for six microsatellite loci[a].

Locus	Primer (5′ to 3′)	Clone repeat sequence	Number of alleles
Pel1	CGGTGATGCTCAAACTTCGT CCTTTCCTACCATTTTTGAA	(GA)4GGAGAA(GA)3A(GA)2	1
Pel2	TAACGCAATCAGTTTATCAA CACACTATTTATGTTCAAGA	(GT)15(GA)11	5
Pel3	AGAGATGGACTGGAAACTTC CCTCCTTAGATTTCTTGTCT	(TC)14(TA)13	6
Pel5	TCTCTGCACACAGGAACCCTTTTGC CCCAGAAATAAGGCTCTTTTGCACA	(AAAG)6	5
Pel6	TCTTATACAATTACAAAAGAAAAGGTG CCTACCTCTCCTTTATATCTACTCTTT	(CT)12(CA)13(TA)2(CA)9	15
Pel7	TGGGGGTAAGTAGAAGAGATTATCG AATATGCCTTCTTTTTGTTTGATTT	(TG)17	5

[a] Data for *Pel1* to *Pel6* are from Chase, Kesseli, and Bawa (1996).

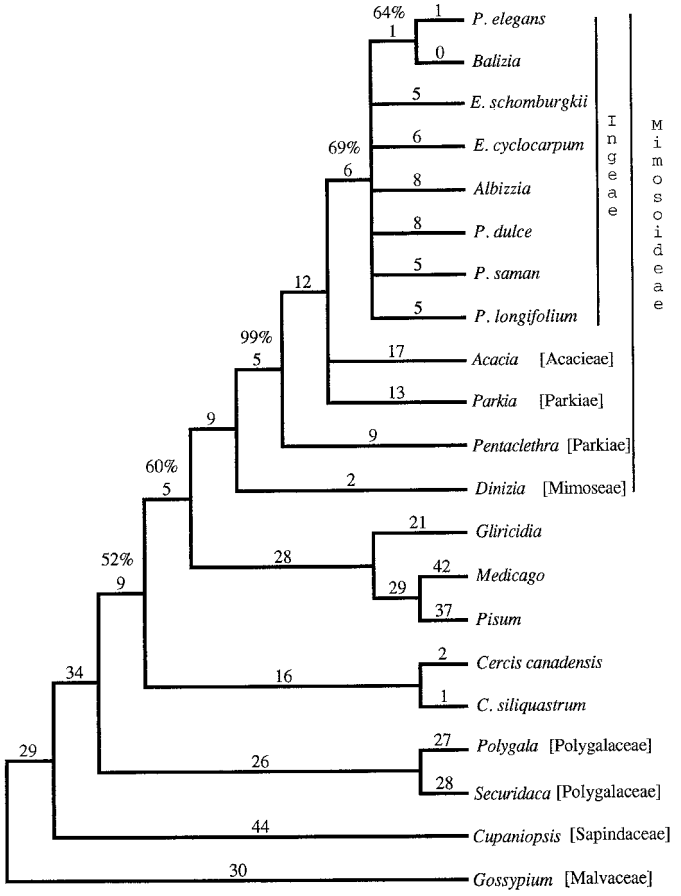

Fig. 1. The strict consensus tree of 73 equally parsimonious trees identified by equally weighted parsimony analysis using *rbcL* sequence data. Length = 514 steps, CI = 0.712, and RI = 0.707. Branch lengths at each node and bootstrap consensus values of branches with <100% are given at each node. Positive amplifications of SSR loci developed from *P. elegans* were observed only among members of the tribe Ingeae.

laceae (Albert, Williams, and Chase, 1992), *Cupaniopsis anacardioides* of Sapindaceae (Chase et al., 1993), *Cercis canadensis, Cercis siliquastrum* of Ceasalpinioideae (Kaess and Wink, 1995), *Pisum sativim* (Zurawski, Whitfield, and Bottomley, 1986) and *Medicago sativa* (Aldrich et al., 1986) of Papilionioideae, and *Gossypium* (Gulov et al., 1990) of Malvaceae were obtained from the Genbank database.

Sequences were aligned using the ClustalW (Thompson, Higgins, and Gibson, 1994) sequence analysis program. Accuracy of sequences was rechecked by translating to peptide sequence and examining for premature stop codons and frame shifts. Aligned sequences were analyzed using PAUP 3.1.1 (Swofford, 1993) and MacClade 3.04 (Maddison and Maddison, 1993) software packages. Parsimony analysis (Kluge and Farris, 1969) was carried out using the branch and bound algorithm (Hendy and Penny, 1982) in the PAUP package with equally weighted nucleotide transformations and default settings (Swofford and Begle, 1993). The *rbcL* sequence of *Gossypium* (Malvaceae) was used as an outgroup. Robustness of resulting trees was tested by bootstrap analysis (Felsenstein, 1985) of 1000 replicates using the heuristic algorithm in PAUP.

RESULTS

Parsimony analysis with equal character weighting resulted in 73 equally parsimonious trees, each with a length of 514 steps, a consistency index (CI) of 0.712, and a retention index (RI) of 0.707. The strict consensus tree is given in Fig. 1. *Gossypium* (Malvaceae), *Cupaniopsis* (Sapindaceae), and *Polygala* (Polygalaceae) were used as outgroups. *Cercis canadensis* and *C. siliquastrum* of the subfamily Caesalpinioideae formed a basal clade to remaining legume species. *Gliricidia, Medicago,* and *Pisum* of Papilionoideae formed a sister clade to the remaining taxa. *Dinizia* was basal to remaining species of Mimosoideae. *Pentaclethra* formed the next clade. *Parkia, Acacia,* and species of the tribe *Ingeae* formed clades sister to each other. The species of Ingeae formed an unresolved clade. Within this group *P. elegans* and *Balizia* clustered together. Bootstrap analysis was in agreement with the strict consensus tree and most of the branches had 100% bootstrap values except the branches between Polygalaceae and Leguminosae (52%), Caesalpinioideae and remaining species of Leguminosae (60%), *Dinizia* and rest of the mimosoids (99%), *Acacia* and *Parkia* clades and *Ingeae* (69%), and the clade with *P. elegans* and *Balizia* (65%).

All primers derived from *P. elegans* could amplify SSR fragments in at least seven related species, though the extent of this success varied markedly (Table 3). At 55°C annealing temperature, all primers except for *Pel 6* showed amplification of bands of comparable size to known alleles of *P. elegans,* in as many as seven related species. Decreasing annealing temperature to 45°C resulted in a positive amplification of *Pel6* as well, with six species (Table 3). Similarly, four species, *Enterolobium schomburgkii, Albizia caribaea, Pithecellobium dulce,* and *P. longifolium,* which did not show positive amplification products with *Pel2* at 55°C annealing temperature showed positive amplification at 45°C annealing temperature. *Balizia excelsa* and *E. schomburgkii* showed amplification with all primers tested. *P. longifolium* showed amplification with all but *Pel6,* whereas *Albizia caribaea* and *P. dulce* showed amplification with all primers except *Pel1*. *Enterolobium cyclocarpum* showed amplification with *Pel5, Pel6,* and *Pel7,* and *P. saman* showed amplifiaction only with *Pel3* and *Pel5.* None of the primers tested showed amplification products with DNA from *Glircidia sepium, Dinizia excelsa, Acacia collincii, Parkia pendula,* and *Pentaclethra macroloba.*

Genetic distances based on divergence of nucleotides in *rbcL* gene from *P. elegans* are given in Table 3. Amplification of SSRs was only observed with species that differ by ten nucleotides or less from the *rbcL* gene of *P. elegans.* No amplification of SSR loci was observed in *Acacia collincii, Parkia pendula, Dinizia, Pentaclethra,* and *Gliricidia,* which showed 21 or more nucleotide position differences from *rbcL* of *P. elegans.* This is also in agreement with the phylogenetic tree obtained through parsimony analysis (Fig. 1) where all species that show positive amplification of SSRs are clustered together.

All loci were polymorphic in at least one other taxon. Considering the narrowness of the germplasm collections for the other species, it was surprising to obtain such high levels of polymorphism. Indeed *Pel1,* a locus monomorphic in *P. elegans,* showed polymorphism in *Balizia.* The other loci that amplified also showed very high levels of polymorphism. Nearly 60% of the species were polymorphic for any given locus and the average number of

TABLE 3. Amplification of microsatellite loci in different species using SSR primers (Pel) developed from *Pithecellobium elgans* DNA[a]. Number of alleles detected in each species for each locus is given. - signifies no amplication products detected; D, number of *rbcL* nucleotide differences as compared to *P. elegans*; AS, number of species that showed amplification for a given locus; PS, number of species that showed polymorphism for a given locus; AL, number of loci that amplified for a given species; PL, number of loci that showed polymorphism for a given species; *N*, total number of individuals tested for each species.

	D	*Pel1*	*Pel2*	*Pel2*	*Pel3*	*Pel5*	*Pel6*	*Pel7*	AL	PL	*N*
Annealing temperature (°C)		55	55	45	55	55	45	55			
Taxon											
Pithecellobium elegans[a]	0	1	5	5	6	5	15	5	6	6	52
Balizia	1	3	3	3	4	2	4	1	6	5	4
Enterolobium schomburgkii	7	1	—	1	1	1	3	1	6	1	4
Pithecellobium saman	7	—	—	—	—	1	—	2	2	1	4
Pithecellobium longifolium	7	1	—	4	3	1	—	2	5	3	4
Enterolobium cyclocarpum	8	—	—	—	—	1	3	2	4	3	4
Pithecellobium dulce	10	1	—	2	6	2	1	1	6	4	6
Albizia caribaea	10	—	—	4	3	2	1	1	5	3	2
Parkia pendula	21	—	—	—	—	—	—	—	0	0	4
Dinizia	24	—	—	—	—	—	—	—	0	0	4
Acacia collincii	25	—	—	—	—	—	—	—	0	0	4
Pentaclethra	27	—	—	—	—	—	—	—	0	0	4
Gliricidia	73	—	—	—	—	—	—	—	0	0	4
Number of species amplified (AS)		4	1	5	5	7	5	7	7	7	
Number of polymorphic species (PS)		1	—	4	4	3	3	3			

[a] Data from Chase, Kesseli, and Bawa (1996).

alleles identified per haploid genome scored was 0.29. That is, for every ten haploid genotypes sampled from a given species, on average, we could identify three alleles per locus. This is higher than results obtained in previous studies of populations of *P. elegans* using *Pel2*, *Pel3*, *Pel5*, and *Pel6* (Chase, Kesseli, and Bawa, 1996). Relatively low levels of variability were detected in two species (*P. saman* and *E. schomburgkii*), while high levels were observed in the remaining taxa. These differences could be due to the nature of the collections (possibly from half-sib arrays) or by sampling error.

DISCUSSION

All primers derived from *P. elegans* showed successful amplification of SSR in other taxa, including species from genera other than *Pithecellobium.* Since phylogenetic relationships of many tropical legumes are not well understood and taxonomic treatments are constantly being revised (Polhill and Raven, 1981; Barneby and Grimes, 1996), we have reconstructed the phylogeny of selected species using chloroplast encoded *rbcL* gene sequence data. Although the reconstruction of the phylogeny in this study was not meant for an exhaustive phylogenetic analysis of legumes, but to ascertain the relatedness among taxa as a basis for predicting the utility of SSRs, some enlightening observations regarding species relationships within Leguminosae can be made.

The taxonomic and phylogenetic placement of the Leguminosae as well as phylogenetic relationships within Leguminosae have been a subject of controversy (Cronquist, 1981; Dickison, 1981; Thorne, 1992). Sapindaceae (Thorne, 1992) and Polygalaceae (Chase et al., 1993) are considered to be closely related to Leguminosae. Therefore, we included *rbcL* sequences of both Sapindaceae and Polygalaceae in our analysis and *Gossipium* (Malvaceae) *rbcL* sequence as a distant outgroup. The resulting tree (Fig. 1) is mostly in agreement with the current view on phylogeny of the Leguminosae (Doyle, 1994).

Within Leguminosae, it has been suggested that *Dinizia* may have closer affinity to Caesalpinioideae than to Mimosoideae (M. Nee and R. Barneby, New York Botanical Gardens, personal communication). In our analysis, however, *Dinizia* clusters with Mimosoideae. Caesalpinioideae may be paraphyletic and some taxa, especially the genus *Dimorphandra* of the tribe Caesalpinieae and members of the tribe Casiaea, are considered to be closely related to Mimosoids (Doyle, 1994). Therefore comparative studies using *rbcL* sequence of *Dinizia* with other Caesalpinioideae may be valuable to make any general conclusions on its affinity. Although *Pentaclethra* is considered as a member of the tribe *Parkiae*, *Pentaclethra*, and *Parkia* form a polyphyletic clade, making *Parkiae* an unnatural taxonomic group that warrants further study. While most of the deeper branches of the tree fit well with current views of taxonomic and phylogenetic relationships, the species delineation and relationships at lower level are suspect. *Pithecellobium elegans* seems closer to *Balizia pedicellaris* than to other congeneric species. Interestingly, in a recent taxonomic treatment *Pithecellobium elegans* has been renamed as *Balizia elegans* (Barneby and Grimes, 1996). Although polymorphism in *rbcL* gene is of limited utility in resolving phylogenetic relationships between closely related taxa, our data do suggest that *P. elegans* and *B. pedicellaris* are closely related, and the placement of *P. elegans* within *Balizia* by Barneby and Grimes (1996) is justified. Nucleotide sequence data from a polymorphic gene such as the ITS region of *rDNA* (Baldwin, 1992) may provide further insights into genetic relatedness between *Balizia* and *Pithecellobium.*

Enterolobium spp. and *Albizia* spp. also appear to be closely related to *Pithecellobium.* Some systematists have placed taxa of *Pithecellobium* within the genus *Albizia* (Polhill and Raven, 1981) . It has been suggested that *Albizia* and *Enterolobium* are closely related and may have derived from ancesters of *Pithecellobium* (Mesquita,

1990). The present data certainly indicate close affinities among these genera. However, further analyses will be necessary for a greater understanding of the phylogenetic relationships and taxonomic boundaries of species.

Although all primers derived from *P. elegans* could amplify SSR fragments in other species, it appears that primer sites are conserved only among taxa within the tribe Ingeae. Our results about conserved sequences are somewhat similar to observations that have been made with certain animal groups (Moore et al., 1991; Schlotterer, Amos, and Tautz, 1991; Levine et al., 1995). Microsatellite loci developed from bovine DNA have shown higher level of conservation in sheep than in horses but are not conserved in humans (Moore et al., 1991). Likewise, the primers for SSR loci characterized from chicken DNA have been used to amplify SSR loci in turkey (Levine et al., 1995). Fitzsimmons, Moritz, and Moore (1995) reported SSR loci in turtles that are found to be conserved across families and Glenn et al. (1996) reported a similar finding for crocodilian genera. The utility of heterologous primers for the amplification of SSRs has also been shown for closely related conspecific or interfertile taxa of plants. Kijas, Fowler, and Thomas (1995) have shown that two sets of primers derived from a *Citrus* × *Poncirus* hybrid could amplify SSRs in these interfertile genera. Similarly, conservation of primers was shown for closely related species in the rice genus *Oryza* (Wu and Tanksley, 1993).

Our study extends this type of analysis to higher taxonomic levels and is among the first plant study to evaluate the allelic diversity in taxa other than that for which the primers were designed. The true utility of SSR loci across taxa depends on the variability of a given locus. Although our samples have limitations, all species and genera within the tribe Ingeae were polymorphic for at least one of the six loci and most taxa examined were polymorphic for three or more loci. In contrast to Fitzsimmons, Moritz, and Moore (1995), who found that heterozygosity is consistently higher in species from which the primers were designed, we have found that polymorphism levels of certain loci are relatively high in related taxa. A new allele for every diploid genotype scored was found for several species–locus combinations. This variation plus the polymorphism found for previously monomorphic *Pel1* was as good or better than that observed in *P. elegans.*

Different primers show different levels of conservation across taxa and different levels of polymorphisms within these related taxa. Previously we had noted that within *P. elegans,* as others have noted in other species, there is a positive relationship between the length of the repeat and the level of polymorphism; *Pel1* with interupted short repeats was monomorphic, while *Pel6* with a compound long repeat was highly polymorphic (Chase et al., 1996). We did not observe a negative relationship between the number of alleles identified in the species from which the primers were derived and the number of taxa that could be amplified with those primers as was noted by Glenn et al. (1996) in their study of crocodilian species. Our data show that there is a threshold genetic distance after which no amplification was observed. We do observe a marginal relationship between the CG content of the primer and the ability of the primer to amplify the DNA of the related taxa. The primers for *Pel5* with the highest CG content (48%) amplified the DNA of all taxa within the tribe Ingeae, while the primers for *Pel2* with the lowest CG ratio (30%) amplified at standard 55°C annealing temperatures the DNA of only one related taxon. Dropping the annealing temperature for this primer pair did elevate to five the number of taxa for which SSRs could be identified.

The data of this study indicate that there is a high potential for the transfer of SSR markers among related taxa. While the conservation of primers for SSR loci was generally limited to the species and genera of the tribe Ingeae, the high variability derived within these species may be unprecedented. If these patterns hold, it is likely that our characterized primers could identify SSRs in hundreds of related species. This utility may vary among major groups, but the potential shown here suggests that considerable time and resources might be saved when primers are available from closely related species. Previously, rapid access to highly polymorphic SSR loci was restricted to species with large DNA databases. The transfer of SSR primers from related species should extend this access and will facilitate the use of SSR loci in a wide variety of genetic analyses related to paternity, mating systems, and gene flow (Terauchi and Konuma, 1994; Chase, Kesseli, and Bawa, 1996; Chase et al., 1996).

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