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THE APPLICATION OF SSRS CHARACTERIZED FOR GRAPE (*VITIS VINIFERA*) TO CONSERVATION STUDIES IN VITACEAE[1]

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The use of microsatellite loci developed from a single plant species across a number of related taxa is becoming increasingly widespread. This approach can provide highly informative markers even for species for which microsatellites have not been characterized. As a number of expressed sequence tag (EST)-derived and enrichment-derived microsatellites are available for grape (*Vitis vinifera*), this study set out to assess transferability of nine such loci across 25 species from five different Vitaceae genera. Intergeneric transfer success in Vitaceae was high (51.1%) and EST-derived loci performed better than enrichment-derived loci. Six loci were further tested across two Australian native species, *Cissus hypoglauca* and *C. sterculiifolia*, in order to assess the conservation of microsatellite repeats and their flanking sequences. Polymorphism of these selected loci was successfully tested for each species across a small, isolated rain forest population from northern New South Wales (Australia). Results from this preliminary investigation suggest that it is possible to use grape-derived simple sequence repeats (SSR) loci for population studies on Vitaceae. As Vitaceae are an important component of rain forest flora, the availability of such highly informative loci will be beneficial to future studies aimed at determining the genetic consequences of rain forest fragmentation.

Key words: *Cissus*; microsatellites; SSR loci transfer; *Vitis*; Vitaceae.

Rain forest habitats are increasingly threatened by anthropomorphic pressures. In northeastern New South Wales (Australia) much of the local subtropical rain forest has been cleared for agricultural purposes. An area known as the ‘‘Big Scrub,’’ originally the largest continuous tract of subtropical rain forest in Australia, is now reduced to <500 ha of small, isolated fragments. Fragmentation of habitat results in modification of gene flow thus affecting the capacity to effectively manage these habitats in the long term (Young, Boyle, and Brown, 1996). Understanding the consequences of these changes, at least within a number of indicator species, is a prerequisite for the preservation of biodiversity. As vines are commonly found within disturbed as well as climax rain forest habitats (Laurance, 1991; Malcolm, 1994; Viana et al., 1997), Vitaceae represent a very useful study group. Vitaceae are woody climbers comprising 13 named genera and ~700 species worldwide (Mabberley, 1995). The family is characterized by leaf-opposed tendrils, which may be modified to form an inflorescence. The most widely recognized species is *Vitis vinifera*, extensively used for the production of fruit and wine. The most speciose genus of the family is *Cissus*, with overall numbers varying according to different taxonomic (Galet, 1967; Mabberley, 1995) and phylogenetic studies (Rossetto et al., 2001). Apart from *Vitis* L., *Ampelopsis* Michx., and *Parthenocissus* Planch., genera mainly restricted to temperate areas, this family has a predominant tropical and subtropical distribution, and its members often play an important role in rain forest habitats and vine thickets worldwide.

Accurate interpretation of genetic diversity within and between populations relies on the choice of appropriate investigative tools. Of the many molecular techniques available to researchers, microsatellites, or simple sequence repeats (SSRs), are becoming increasingly widespread. These markers are codominant, frequently and evenly distributed throughout the genome, selectively neutral, highly reproducible and rely on simple polymerase chain reaction (PCR) technology. Slipped-strand mispairings during DNA replication accumulate more rapidly than point mutations and indels and therefore produce a high number of alleles per locus (SSR mutation rates have been estimated at 10^{-2} to 10^{-3} per locus per gamete per generation; Weber and Wong, 1993). Such highly polymorphic behavior is particularly useful when investigating extent of gene flow, patterns of differentiation, and levels of inbreeding among populations. The major constraint to a more extensive adoption of SSRs in conservation studies is the lack of available loci. Despite the development of efficient enrichment techniques (e.g., Edwards et al., 1996; Fisher and Bachmann, 1998), many laboratories have sufficient resources and expertise for SSR-based PCR analysis but not for the isolation and characterization of new loci. One possible solution is the use of previously developed SSR primers to detect polymorphism at corresponding loci in related target species. Success of heterologous PCR amplification is likely to depend upon evolutionary distances between source and target species. Higher genomic homology should translate into greater conservation of SSR flanking regions and, as a result, transferability of primer pairs. The availability of a large number of SSR loci for economically important species has increased interest in primer transferability, at least across closely related taxa.

As a major horticultural crop, *Vitis vinifera* has been the target of much research including genetic and genomic studies. Grape microsatellites have been developed for a variety of applications such as mapping, genotyping, and breeding (Thomas and Scott, 1993; Bowers et al., 1996; Scott et al., 2000). Recently, a gene discovery project in *V. vinifera* produced a database of 5000 expressed sequence tags (ESTs)

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TABLE 1. List of the 25 taxa used for the cross species test and their location of origin (NSW = New South Wales; Qld = Queensland; WA = Western Australia).

Genus	Species	Provenance
Cayratia	*acris* (F.Muell.) Domin.	Millaa Millaa (Qld)
	cardiophylla Jackes	Chillagoe (Qld)
	clematidea (F.Muell.) Domin.	Lismore (NSW)
	eurynema B.L.Burtt	Lismore (NSW)
	maritima Jackes	Cairns (Qld)
	saponaria (Seem. Ex Benth.) Domin.	Millaa Millaa (Qld)
	trifolia (L.) Domin.	Townsville (Qld)
Cissus	*adnata* Roxb.	Weipa (Qld)
	antarctica Vent.	Lismore (NSW)
	cardiophylla (F.Muell) Jackes	Harvey Range (Qld)
	hastata Miq.	Townsville (Qld)
	hypoglauca A.Gray	Lismore (NSW)
	oblonga (Benth.) Planch.	Harvey Range (Qld)
	opaca F.Muell.	Mt Fox (Qld)
	penninervis (F.Muell.) Planch.	Paluma (Qld)
	reniformis Domin.	Magnetic Island (Qld)
	repens Lam.	Tully (Qld)
	sterculiifolia (F.Muell. ex Benth) Planch.	Lismore (NSW)
	vinosa Jackes	Ingham (Qld)
Clematicissus	*angustissima* (F.Muell.) Planch.	Kalbarri (WA)
Tetrastigma	*crenatum* Jackes	Cardstone (Qld)
	nitens (F.Muell.) Planch.	Townsville (Qld)
	petraeum Jackes	Mareeba (Qld)
	thorsborneorum Jackes	Magnetic Island (Qld)
Vitis	*riparia* Michx.	unknown
	vinifera L.	Pinot Noir (Tasmania)

(Ablett et al., 2000) from which 124 repeat sequences were discovered (Scott et al., 2000). The successful transfer of grape SSRs across a number of Vitaceae would provide useful tools for the appraisal of population genetic parameters within fragmented rain forest populations.

In this study, the transfer potential of nine grape SSR loci (six EST-derived and three enrichment-derived) was assessed across the main Vitaceae genera present within Australian rain forests. An initial experimental stage consisted in assessing amplification success across 25 species. Based on the success of this preliminary stage, a second experimental stage consisted of a more precise assessment of grape SSR transferability across two species (*Cissus hypoglauca* and *C. sterculiifolia*) commonly found throughout the “Big Scrub” remnants. Amplification products were sequenced for six loci to verify the presence of SSR repeats, and polymorphism was assessed across a small, isolated population from which little variability was expected. If these grape-derived SSRs prove to be transferable and polymorphic in the study species, they will provide useful tools for gene-flow studies in fragmented populations and overcome the need for species-specific libraries.

MATERIALS AND METHODS

Plant material—Twenty-four Australian Vitaceae species were used to test cross transferability of grape SSRs across genera (Table 1). A single individual per species was tested for most species, as it was the only material accessible. Since assessing transferability rather than polymorphism was the main objective of the initial stage of this study, this approach was considered as satisfactory. *Vitis riparia* was included as a within-genus comparison.

In the second stage of this study, *Cissus hypoglauca* and *C. sterculiifolia* were selected as test species because of their wide distribution within the area of interest, the “Big Scrub.” *Cissus hypoglauca* is found in rain forest habitats from eastern Victoria to north of the Daintree River (northern Queensland; Jackes, 1988). *Cissus sterculiifolia* is found in rain forest habitats from south of Sydney (New South Wales) to the Atherton Tableland (northern Queensland). In order to test polymorphism of the six selected loci a small isolated fragment of coastal rain forest containing both species was chosen. Fourteen and 15 individuals were sampled for *C. hypoglauca* and *C. sterculiifolia*, respectively. The potential presence of distinct alleles within distant populations was also assessed by assaying three northern Queensland individuals for each species.

DNA extractions and PCR analysis—The plant material used in the initial stage was either collected and extracted fresh or was of herbarium origin. All material used in the second experimental stage was collected in situ and extracted fresh. Total DNA was extracted using a protocol previously described by Rossetto et al. (2001).

The PCR amplifications were performed in 25-μL reaction volumes containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 0.5 unit Taq polymerase (Roche), 0.2 mmol/L of each dNTP, 5 μmol/L of each forward and reverse primer, 25 ng of template DNA plus DNA-free water. The PCRs were run under the following conditions: an initial denaturation step of 94°C for 4 min, followed by 30 cycles of 94°C for 30 sec, an annealing temperature of 48°C–60°C depending on the primer pair used (Table 2) for 30 sec and an extension at 72°C for 30 sec, with a final extension step at 72°C for 7 min prior to holding at 4°C. In order to make this study simple and efficient, if cross-species amplification was not satisfactory at the recommended annealing temperature, the principal approach was to decrease this temperature. The EST-derived primers used are those described by Scott et al. (2000), whereas the library-derived primers are previously unpublished (Table 2) and were characterized using Edwards’ et al. (1996) enrichment technique.

The PCR products were first run on a 2% agarose gel and visualized with ethidium bromide in order to detect amplification success. If amplification was successful, the PCR product was run on a laser-scanning polyacrylamide gel system, the Gel-Scan 2000 (Corbett Research, Sydney, Australia), and visualized with ethidium bromide in order to assess if the fragment obtained was within the expected size range. Samples were run on an 18 cm long, 0.1 mm thick 5% polyacrylamide nondenaturing gel. Gels were pre-run at 1200 V, 40°C for 20 min; samples were loaded and run under the same conditions.

TABLE 2. Sequence details for the ten primer pairs used in this study. The SSR repeat type and size (number of base pairs) in the original *V. vinifera* fragment are indicated. Original PCR annealing temperature and temperature test ranges are also indicated. The enrichment-derived primers (*vmc8A7*, *vmc8B12*, *vmc8D11*) are previously unpublished.

Locus	Primer sequences (5′ to 3′)	SSR repeat	Original size (bp)	Annealing temperature	Tested temperature range
scu05vv	fwd: CAAGCAGTTATTGAAGCTGCAAGG rvs: TCATCCATCACACAGGAAACAGTG	AT	174	51.2°C	48°–55°C
scu06vv	fwd: CCTAATGCCAGGAAGGTTGC rvs: CCCTAGTCTCTCTACCTATCCATG	AT	171	49.7°C	45°–55°C
scu08vv	fwd: CGAGACCCAGCATCGTTTCAAG rvs: GCAAAATCCTCCCCGTACAAGTC	GGT	180	57.7°C	48°–55°C
scu11vv	fwd: AATTGATAGTGCCACGTTCTCGCC rvs: ACGCCGACAAGAATCCCAAGG	CTT	248	57.3°C	48°–55°C
scu14vv	fwd: CTGCACTTGAATACGAGCAGGTC rvs: TGTTATATGATCCTCCCCCTCCTC	GAA	182	53.6°C	48°–55°C
scu15vv	fwd: GCCTATGTGCCAGACCAAAAAC rvs: TTGGAAGTAGCCAGCCCAACCTTC	GAA	195	53.5°C	48°–55°C
scu16vv[a]	fwd: CAAAGACAAAGAAGCCACCGAC rvs: ACCCTCTAAAGCACACACAGGAAC	GAA	170	54.4°C	48°–55°C
vmc8A7	fwd: GCAGCAACTCTCTTACACACCG rvs: GTGGGAGCACTGGTTGCTTTAG	TC	163	64.5°C	48°–60°C
vmc8B12	fwd: AGAGCACGCTGGACCTTCTTC rvs: GCACTGCGCGATTTCACTC	TC	142	65.0°C	48°–60°C
vmc8D11	fwd: TGTTGAAGCTAGCATTGTCTCC rvs: ATTCGTCTTTATGCCCATTGTT	GA	143	61.0°C	48°–60°C

[a] This primer pair was only used in the *Cissus* variability test.

Gene Profiler (Scanalytics, Fairfax, Virginia, USA) was then used for accurate sizing, allowing single base differences to be detected.

Amplification product cloning and sequencing—To confirm the amplification of the expected SSR repeats, the amplification products obtained for six loci (*scu05vv, scu06vv, scu08vv, scu15vv, scu16vv, vmc8D11*) were sequenced for *C. sterculiifolia* and *C. hypoglauca*. The PCRs that produced a single allele could be sequenced directly (forward and reverse), whereas the individuals that produced heterozygous patterns required cloning. The pGEM-T Easy Vector Systems (Promega, Madison, Wisconsin, USA) was used for cloning following the manufacturer's instructions. The cloned fragments were sequenced using the ABI Prism Big Dye terminator cycle-sequencing kit (Perkin Elmer Applied Biosystems, Foster City, California, USA) according to the manufacturer's directions. Visualization of sequence data was carried out on ABI 377 sequencers at the Australian Genome Research Facility (Brisbane, Queensland, Australia). The sequences obtained were aligned using Clustal W (Thompson, Higgins, and Gibson, 1994).

RESULTS

Stage one: amplification of grape SSRs across Vitaceae—A total of nine loci were assessed across 25 species for a total of 225 individual primer/species combinations. Of these, 115 (51.1%) produced fragments within the expected size range. A further 35 (15.5%) combinations also produced fragments, but outside the expected size range (i.e., $>$100 base pairs [bp] larger or smaller than the original *V. vinifera* sequence). This range was arbitrarily selected to simplify the analysis (especially in the cases where more than two bands were amplified) and does not necessarily imply that the repeat sequence was absent. All successful amplifications were obtained at the recommended primer annealing temperature or at slightly lower temperatures (see Table 2 for details of the ranges tested). More complex PCR protocols, such as touchdown, did not significantly improve amplification success.

Success across the different loci varied from 96% at locus *scu15vv* to 36% at locus *vmc8B12* (Table 3). The SSR loci derived from the enriched library (*vmc8A7, vmc8B12, vmc8D11*) successfully amplified products within the expected size range in 42.7% of all combinations tested. Those derived from the EST database were more efficient, with 56% success. No comparison based on repeat type could be made on the enrichment-derived microsatellites, as all those tested were dimers. However, within the EST-derived SSRs, 46% of the combinations involving dinucleotide repeats (*scu05vv, scu06vv*) were successful as opposed to 61% for those involving trinucleotide repeats.

When assessing the results across species, locus transferability varied from eight out of nine for *V. riparia*, *C. acris*, and *T. petraeum* to a single successful amplification for a number of species (usually *scu15vv* being the successful locus; Table 3). However, some of the negative results for locus *scu15vv* might have been caused by the poor DNA quality of some herbarium-derived samples. Across genera, success was very high for *Tetrastigma* (75%), medium for the monotypic genus *Clematicissus* (55.6%) and for *Cissus* (46.3%), and lower for *Cayratia* (39.7%). Good transfer success for *Cissus hypoglauca* and *C. sterculiifolia* (77.8%) was of interest, as these species were shown to be distinct from the genus *Cissus* in a recent phylogenetic study (Rossetto et al., 2001). Overall, this initial stage suggests that these highly informative markers, originally developed for grapes, are likely to be accessible to a large number of related species.

Stage two: sequence conservation and polymorphism of grape SSRs across two selected species—In order to ensure that the desired loci were amplified in *C. hypoglauca* and *C. sterculifolia*, the PCR product obtained with six primer pairs was sequenced. Figure 1 shows sequence conservation for the six primer/species combinations investigated (*scu05vv, scu06vv, scu08vv, scu15vv, scu16vv, vmc8D11*). Forward and reverse primer sequences were clearly conserved across taxa, except for a single deletion in the reverse primer of locus *scu16vv* in *C. sterculiifolia* (Fig. 1). The SSR repeat type was also retained in all species at all loci; however, interruptions

TABLE 3. Results from the SSR transferability test on the 25 Vitaceae species tested. The first six columns represent the results for the EST derived SSRs (obtained from a Chardonnay EST library) and the last three enrichment derived ones (obtained from a Shiraz SSR library). A + indicates successful amplification within the arbitrary expected size range (i.e., 100 bp above or below the original *Vitis vinifera* fragment), w indicates amplification outside the expected range, and − indicates no amplification.

		Loci								
Genus	Species	*scu11vv*	*scu08vv*	*scu14vv*	*scu15vv*	*scu06vv*	*scu05vv*	*vmc8D11*	*vmc8B12*	*vmc8A7*
Cayratia	*acris*	+	+	+	+	+	+	+	w	+
	cardiophylla	+	+	w	+	−	+	−	w	+
	clematidea	−	−	−	+	−	−	−	w	−
	eurynema	w	w	−	+	−	−	−	w	w
	maritima	−	w	−	+	−	−	−	−	−
	saponaria	−	+	+	+	+	−	+	+	+
	trifolia	−	−	+	+	−	−	−	−	−
Cissus	*adnata*	−	−	+	−	−	−	−	−	−
	antarctica	+	−	−	+	−	−	+	w	−
	cardiophylla	w	w	+	+	+	+	w	+	−
	hastata	w	w	−	+	+	−	−	w	−
	hypoglauca	+	+	+	+	+	+	+	w	−
	oblonga	+	−	−	+	+	−	+	+	+
	opaca	w	−	+	+	−	−	+	+	+
	penninervis	+	+	−	+	+	−	+	+	−
	reniformis	w	w	−	+	−	−	−	w	−
	repens	+	w	+	+	w	+	+	+	+
	sterculiifolia	+	+	+	+	+	+	+	w	−
	vinosa	w	−	−	+	−	−	−	w	−
Clematicissus	*angustissima*	+	w	+	+	−	−	+	w	+
Tetrastigma	*crenatum*	+	+	+	+	+	−	w	w	−
	nitens	w	+	+	+	+	+	w	+	+
	petraeum	w	+	+	+	+	+	+	+	+
	thorsborneorum	+	+	w	+	+	+	−	+	+
Vitis	*riparia*	+	+	+	+	+	+	+	w	+

were present in some instances (Fig. 1). Such interruption can potentially reduce the mutational capacity of the SSR, as imperfect repeats are traditionally considered to be less polymorphic. Interestingly, a considerable amount of variability, including indels and base pair substitutions, was detected within the SSR flanking regions. Consequently, allele length variation between species was affected by SSR size as well as the presence of indels within the flanking region (Fig. 1). Differences from the original *V. vinifera* sequence were often shared by the two native species, with some notable exceptions, such as a 12-bp insertion at locus *scu16vv*, present only in *C. sterculiifolia* (Fig. 1). Of the 39 substitutions and indels in the flanking regions of the six loci, only 18% were unique to *C. sterculiifolia* and 10% to *C. hypoglauca*.

The potential of these six loci to detect polymorphism was tested across individuals from a small, isolated rain forest population from northern New South Wales. A total of 21 and 22 alleles were detected for a limited number of individuals for *C. hypoglauca* and *C. sterculiifolia* respectively (Table 4). A further three individuals from northern Queensland were tested in both species in order to assess the presence of novel alleles. Four and two novel alleles were amplified from these small outgroups of *C. hypoglauca* and *C. sterculiifolia*, respectively.

DISCUSSION

The prospect of using SSRs across more than one species is likely to make this technology more accessible to a broader range of investigations. In particular, population and conservation studies are likely to benefit from this approach as the number of loci required is usually smaller than for mapping and breeding research. Yet to date, only a limited number of plant population studies have relied on the application of SSR loci characterized in related species (e.g., Strieff et al., 1998; Degen, Strieff, and Ziegenhagen, 1999). Our data suggest that *V. vinifera* SSR loci (especially EST-derived loci) successfully transfer across other Vitaceae genera. Further investigation on sequence conservation revealed that at least six loci were fully preserved and produced polymorphism across small, isolated populations of two rain forest Vitaceae. Thus, for *C. hypoglauca* and *C. sterculiifolia*, the use of grape SSRs is a viable alternative to the development of species-specific libraries in the study of the effects of rain forest fragmentation.

Transfer success of grape SSRs across Vitaceae—Transfer of grape SSR loci across Vitaceae was more successful than similar studies in other families. A previous review of SSR cross-transferability in plants (Rossetto, 2001) showed that average intergeneric success was 35.2%, considerably lower than the 51.1% obtained within Vitaceae. The data in Table 3 suggests that the evolutionary relationship between target and source species may play an important role in SSR transfer success. Locus amplification success differed among genera and appeared to be predominant in taxa more closely related to grapes, such as *V. riparia*, *hypoglauca*, and *C. sterculiifolia*, for example (Table 3). A recent cpDNA-based study showed that *C. hypoglauca* and *C. sterculiifolia* were not true *Cissus* but part of a new, distinct clade possibly related to *Vitis* (Rossetto et al., 2001). Phylogenetic reconstructions purely based on SSR alleles (i.e., on repeat sequences) are considered ambiguous mainly because of restrictions in repeat range, unevenness of mutational processes, and gradual degradation of the repeat sequence. Nevertheless, if number and type of SSR alleles are unlikely to make a real contribution to phylogenetic reconstructions, the diversity within their flanking regions may provide a considerable amount of useful information (Fig. 1).

scu05vv

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V. inifera         1 CAAGCAGTTATTGAAGCTGCAAGGAATGTCCTGGAGTCTCGTAAAGATAAAGTTCAGAGTGACCCACATCCGGAGGCAGCTGCATTTATC
C.sterculiifolia   1 ........................................------.......G................TT...........GA.....
C.hypoglauca       1 ........................................------.......G................TT.......T....A.....


V.vinifera        91 -TAATAGGG-AATGCTATTTTTACAATATATATATATATATATATATATATATTTTCAGTTTTTCACTGTTTCCTGTGTGATGGATGA 174
C.sterculiifolia  91 .........C...........C....C.........G.G...G.........C-................................ 168
C.hypoglauca      91 A........C............C.............G.G...G..........-................................ 169
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scu06vv

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V.vinifera         1 CCTAATGCCAGGAAGGTTGCTAGCACTTGTGGGACTCCCATCCCTAAGTGCTAGATAA---CTAGATTTTAAA---------TTTTCTTC
C.sterculiifolia   1 .......................................G.................GTGA...........GGCACTCGTT........
C.hypoglauca       1 .......................................G.................GTGA...........GGCACTCTTT........


V.vinifera        91 TCTATGTCTCTCTTTCTAGATATATATATATATATAAATATCCTCTGCATGCATCTTTCTATATAGTTTCATGGATAGGTAGAGAGACTA
C.sterculiifolia  91 ...................----------.....................C.......................................
C.hypoglauca      91 ...................----------...................T.T.......................................


V.vinifera       181 GGG  171
C.sterculiifolia 181 ...  173
C.hypoglauca     181 ...  173
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scu08vv

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V.vinifera         1 CGAGACCCAGCATCGTTTCAAGTGTTTCTGCTACTTCTTC---AGGAAATTCTGAGCTCCGGTGGTGGTGGTGGT------GATGGAGAG
C.sterculiifolia   1 .................................T..A...TCT......G.........................GGTGGT.........
C.hypoglauca       1 .................................T......TCT......G...................------------.........


V.vinifera        91 CACGTTGAAGGACATAAGAGATGGCGCTTCGGTGCTGGACCTGGACCCAAAGGCCACCGTGGGTGGTGGCGTTGAGGACTTGTACGGGGA
C.sterculiifolia  91 T........A.....C..........................................................................
C.hypoglauca      91 T........A................................................................................


V.vinifera       181 GGATTTTGC   180
C.sterculiifolia 181 .........   189
C.hypoglauca     181 .........   177
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scu15vv

```
V.vinifera         1 GCCTATGTGCCAGACCAAAAACCCTGACCC--TTGGCAAT-GAGGAAAA-TCTGAAGCACGTCTACAAATTC-TTTTCATTTCAACCAGA
C.sterculiifolia   1 ..............................CC...........A.............T...............A...............G
C.hypoglauca       1 ......................A.......CC........T........A.......T..............CA...............T

V.vinifera        91 AAAGTGAAGAAGAAGAAGAAGAA-------------------GCCTACAAA-GACTAGAAAATAGA--CTCAGAAACCCGGTTCAACCAA
C.sterculiifolia  91 T.T.............................GAAGAAAGAA.........A..............GA......................
C.hypoglauca      91 .......................GATGATGAAGAAGAAAGAA.........A.....T...C....GA......................

V.vinifera       181 ATTTAAAGCCACCTGGAGGAAGGTTGGGCTGGCTACTTCC   193
C.sterculiifolia 181 ........................................   207
C.hypoglauca     181 ........................................   210
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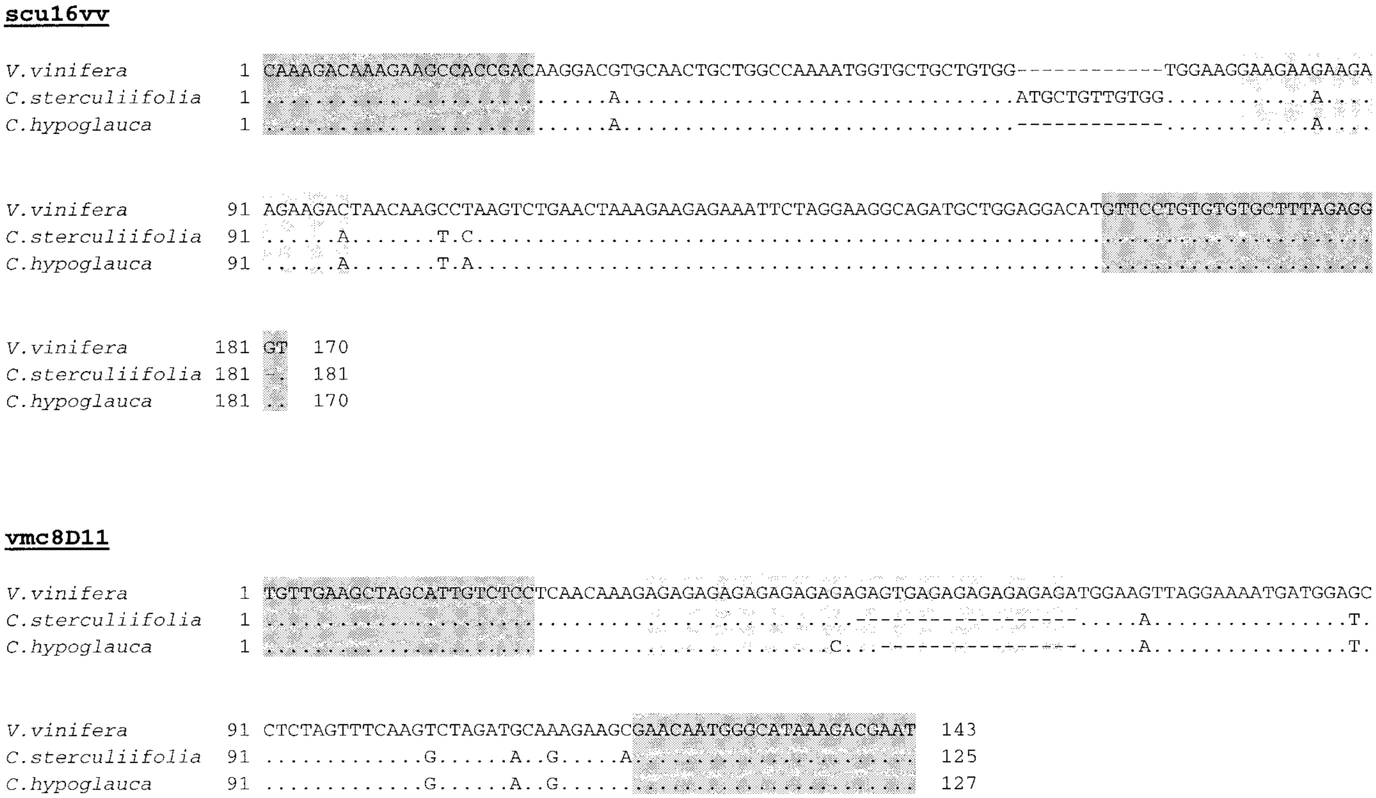

```
scu16vv

V.vinifera        1 CAAAGACAAAGAAGCCACCGACAAGGACGTGCAACTGCTGGCCAAAATGGTGCTGCTGTGG------------TGGAAGGAAGAAGAAGA
C.sterculiifolia  1 ............................A................................ATGCTGTTGTGG............A....
C.hypoglauca      1 ............................A................................------------............A....

V.vinifera       91 AGAAGACTAACAAGCCTAAGTCTGAACTAAAGAAGAGAAATTCTAGGAAGGCAGATGCTGGAGGACATGTTCCTGTGTGTGCTTTAGAGG
C.sterculiifolia 91 ......A.......T.C.........................................................................
C.hypoglauca     91 ......A.......T.A.........................................................................

V.vinifera      181 GT  170
C.sterculiifolia 181 -.  181
C.hypoglauca    181 ..  170
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vmc8D11

V.vinifera        1 TGTTGAAGCTAGCATTGTCTCCTCAACAAAGAGAGAGAGAGAGAGAGAGAGTGAGAGAGAGAGAGATGGAAGTTAGGAAAATGATGGAGC
C.sterculiifolia  1 ................................................------------------.....A................T.
C.hypoglauca      1 ...............................................C..----------------.....A................T.

V.vinifera       91 CTCTAGTTTCAAGTCTAGATGCAAAGAAGCGAACAATGGGCATAAAGACGAAT  143
C.sterculiifolia 91 .............G.....A..G.....A........................  125
C.hypoglauca     91 .............G.....A..G..............................  127
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Fig. 1. Continued.

An EST-derived characterization of microsatellites relies on the existence of databases of expressed DNA regions, while enrichment-derived characterization is based on randomly sampling the entire genotype. The data in Table 3 suggest that EST-derived SSRs transferred across genera more readily (55.3%) than enrichment-derived SSRs (42.7%). Previous EST-based studies have been less successful, possibly because they aimed at genotyping germplasm collections with fairly low diversity (e.g., Brown et al., 1996). Good transferability of EST-derived loci should not come as a surprise, since evolutionary constraints within coding sequences should limit mutational events and increase sequence similarity. However, excessive conservation can have negative consequences, and EST-derived SSR loci are generally expected to display low polymorphism. Interestingly, in this study, the most conserved locus across genera, *scu15vv* (Table 3), was also the most polymorphic (Table 4). The polymorphism of *scu15vv* and the high levels of variability detected within the SSR flaking regions (Fig. 1) suggest that at least some SSR-containing expressed sequences are more flexible in structure than anticipated. These findings indicate that mining EST databases for SSR sequences might be a useful approach for species related to economically important taxa.

Grape-derived SSR loci for population studies within wild relatives—The potential advantages of SSR cross transferability are numerous. Lengthy and expensive enrichment procedures can be avoided, thus making this technique available to a greater range of organisms and applications. Furthermore, the use of identical loci across different species allows for direct comparison of the results without the need for assumption on the evolutionary rates of the various loci. Researchers working on species related to taxa for which large databases (SSR and/or EST) are available in the public domain are particularly advantaged. These may still be a minority, but as gene discovery becomes increasingly approachable, opportunities for further investigations will multiply. In the future, increased cooperation between agricultural and conservation research should ensure that new findings are easily accessible to a greater range of projects.

This study intended to investigate the application potential of *V. vinifera* SSRs to other Vitaceae. In particular, highly informative markers were required for future investigations on the effects of isolation and fragmentation of rain forest habitats. It is, however, important to bear in mind that comparing SSR products obtained in distantly related species should be approached with caution. Various factors can cause size homoplasy, and thus the amplification of a PCR product does not necessarily imply locus conservation. As a result of this possible confusion, it has previously been suggested that PCR fragment identity should be verified by sequencing (Peakall et al., 1998; Rossetto et al., 2000). As the species selected in this

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Fig. 1. Sequence alignments for five EST-derived (*scu05vv, scu06vv, scu08vv, scu15vv, scu16vv*) and one enrichment-derived (*vmc8D11*) *Vitis vinifera* SSR loci, amplified in *Cissus hypoglauca* and *C. sterculiifolia*. The forward and reverse primers regions are dark shaded and the repeat sequences are light shaded. Indels and substitutions are marked.

TABLE 4. Polymorphism data from one small isolated population in northern New South Wales of *Cissus hypoglauca* and *C. sterculiifolia*. The number of individuals tested per species and the number of alleles obtained with each locus are indicated. Expected heterozygosity H_e is also shown.

Species	N	Loci						H_e
		scu05vv	*scu06vv*	*scu08vv*	*scu15vv*	*scu16vv*	*vmc8D11*	
C. hypoglauca	14	3	2	2	7	2	5	0.50
C. sterculiifolia	15	3	6	3	5	3	2	0.58

study (*C. hypoglauca* and *C. sterculiifolia*) belong to a different genus from the one for which the SSR library was developed, sequencing was successfully used to support the amplification data (Fig. 1). Such a cautious approach is important when working across genera and particularly if uncertainty exists regarding the size range of the fragments obtained. However, if working within the same genus and if satisfactory amplification products are obtained, such a thorough verification might not be required.

Our results indicate that previously characterized SSR loci can be successfully used in ecological and conservation studies across related species. Grape SSRs are likely to provide useful genetic tools for population investigations of selected Vitaceae worldwide.

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