J. Amer. Soc. Hort. Sci. 123(2):182–188. 1998.

Using Simple Sequence Repeats (SSRs) for DNA Fingerprinting Germplasm Accessions of Grape (*Vitis* L.) Species

Warren F. Lamboy[1] and Christopher G. Alpha[2]

U.S. Department of Agriculture–Agricultural Reearch Service, Plant Genetic Resources Unit, Department of Horticultural Sciences, Cornell University, Geneva, NY 14456-0462

Additional index words. microsatellite markers, identification, discrimination power, genetic resources

Abstract. **The USDA–ARS *Vitis* genetic resources collections in Geneva, N.Y., and Davis, Calif., contain ≈3600 accessions of >35 species. Accurate and unambiguous identification of these grapes is essential for efficient and effective use of this germplasm. Previous workers have successfully used polymerase chain reaction (PCR)-generated SSRs to fingerprint cultivars of the wine and table grape species, *V. vinifera*. Building on this work, we conducted a test of five previously characterized SSR loci on 110 accessions of 25 grape taxa (21 *Vitis* species and 4 hybrids) to determine if they would satisfy our need for identifying cultivars within the USDA–ARS grape collections. Scorable SSR fragments were produced with all 550 primer–accession combinations, with no null loci observed. The loci were highly polymorphic, with 16 to 38 different alleles found at a locus. Heterozygosity values ranged from 0.464 to 0.818, while gene diversity values ranged from 0.875 to 0.955. Discrimination power at a locus varied from a low of 0.947 to a high of 0.987. Combined discrimination power of all loci was effectively 1.000, with 2 chances in 100,000,000 that two sexually, independently derived grape accessions would not be distinguishable using this set of five SSR loci. Two plants in the study that had previously been classified as belonging to different grape species were shown to have identical SSR fingerprints, showing that they almost certainly possessed the same genotype. Because SSR markers are codominant and highly polymorphic and SSR loci are generally conserved across a range of related species, we strongly recommend SSRs for fingerprinting not only grape, but other clonal genetic resources collections as well.**

The *Vitis* genetic resources collections at the USDA–ARS Plant Genetic Resources Unit (PGRU) at Cornell Univ., Geneva, N.Y., and the USDA–ARS National Germplasm Repository (NGR) at the Univ. of California, Davis, together contain >3600 different grape accessions. The PGRU collection has ≈1300 accessions comprising 62% North American and French–American hybrids, 27% non-*V. vinifera* L. species, 7% *V. vinifera* cultivars, and 4% undetermined, while the NGR collection contains >2300 accessions, 27% hybrids, 22% non-*V. vinifera* species, 44% *V. vinifera* cultivars, and 7% undetermined.

Accurate identification of all these accessions is essential if the two collections are to be reliable sources of grape germplasm. Members of the user community typically request germplasm based on traits known to be present in specific species, cultivars, accessions, or genotypes (Kresovich and McFerson, 1992; McFerson et al., 1996; Peeters and Galwey, 1988). Furthermore, correctly identified accessions enable curators to discover and possibly discard duplicates in their collections and prevent the costly reacquisition of genotypes already present.

Ampelography (Galet, 1979, 1988, 1990), a classical method for identifying *V. vinifera* cultivars using morphological characters, is not helpful in identifying accessions of other grape species and their hybrids, which make up 90% of the Geneva collection and 50% of the Davis collection. Even in *V. vinifera*, ampelography is not always reliable; sometimes even expert ampelographers disagree on the identity of a plant (Bowers et al., 1993).

A further difficulty encountered in identifying grapes is the large number—14000 (Alleweldt et al., 1990)—of known cultivars. Correct identification also is hampered by the use of two or more different names for the same cultivar or genotype (Bowers et al., 1996), or even the use of the same name for two or more different cultivars or genotypes (Bowers et al., 1996). A reliable, consistent, and reproducible method for confirming the identities of grape accessions is needed (Alleweldt et al., 1990).

Fortunately, a class of polymerase chain reaction (PCR)-based markers, called microsatellites or SSRs, has already been used successfully for identifying grape genotypes, primarily among *V. vinifera* cultivars (Botta et al., 1995; Bowers et al., 1996; Thomas and Scott, 1993; Thomas et al., 1993, 1994). Their extremely high levels of polymorphism, their codominant inheritance, and the public availability of their primer sequences (Bowers et al., 1996; Thomas and Scott, 1993) make SSRs prime candidates for use in DNA fingerprinting the broad array of genotypes held in the USDA–ARS *Vitis* germplasm collections.

There are two potential limitations to the use of previously discovered SSR loci for this purpose. First, existing primers were developed from *V. vinifera* DNA (Bowers et al., 1996; Thomas et al., 1993, 1994; Thomas and Scott, 1993), and the loci they amplify may

Received for publication 2 May 1997. Accepted for publication 2 Oct. 1997. Mention of a proprietary product does not constitute an endorsement or recommendation for use by the USDA. We are grateful to John Bowers and Carole Meredith of the Univ. of California, Davis, for providing primer sequences and amplification protocols and for fruitful discussions of grape SSRs. We thank Mark Thomas and Nigel Scott of CSIRO, Adelaide, South Australia, for sharing their SSR results, techniques, and data. Steve Kresovich and his collaborators at the USDA–ARS Plant Genetic Resources Conservation Unit, Griffin, Ga., provided enthusiastic encouragement in the use of SSRs for the documentation of genetic diversity in germplasm collections and leadership in using SSRs in germplasm management. Gel electrophoresis was conducted in the labs of Doug Knipple, Dept. of Entomology, Cornell Univ., Geneva, N.Y., and of Steve Kresovich; without their cooperation and assistance, this work would not have been possible. We thank George White and Bernard Prins, USDA–ARS National Germplasm Repository, Univ. of California, Davis, who supplied leaf material of some North American grape species, and Robert Pool, Dept. of Horticultural Sciences, Cornell Univ., who permitted us to collect grape leaf tissue from selected *V. vinifera* cultivars. Stuart Brown, Phil Forsline, Stan Hokanson, Jim McFerson, Cathy Ronning, Amy Szewc-McFadden, Norm Weeden, and two anonymous reviewers critically read the manuscript and provided many helpful comments. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]To whom reprint requests should be addressed; e-mail wfl1@cornell.edu.

[2]Current address: Laboratory for Stable Isotope Research and Analysis, The Boyce Thompson Institute for Plant Science Research, Tower Road, Ithaca, NY 14853.

Table 1. Grape germplasm accessions assayed for SSR polymorphisms.[z]

Accession identification	Accession no.
V. acerifolia	PI 318684
V. acerifolia	PI 588144
V. acerifolia	PI 588442
V. acerifolia	PI 588448
V. aestivalis	PI 588145
V. aestivalis	PI 588182
V. aestivalis	PI 588626
V. amurensis[y]	PI 588382
V. amurensis[y]	PI 588420
V. amurensis[y]	PI 588640
V. arizonica	DVIT 1269
V. arizonica	DVIT 1872
V. arizonica	DVIT 2211
V. californica	DVIT 1275
V. californica	DVIT 1360
V. californica	DVIT 1361
V. californica	DVIT 1836
V. cinerea	PI 588199
V. cinerea	PI 588210
V. cinerea	PI 588220
V. cinerea	PI 588222
V. cinerea	PI 588447
V. cinerea	PI 588460
V. cinerea	PI 588688
V. coignetiae[y]	PI 588058
V. coignetiae[y]	PI 588451
V. girdiana	DVIT 1379
V. girdiana	DVIT 1380
V. girdiana Redlands	DVIT 1387
V. girdiana	DVIT 1389
V. labrusca	PI 588173
V. labrusca	PI 588583
V. labrusca Alba	PI 588165
V. labrusca Alexander	PI 594349
V. labrusca Champagne	PI 588307
V. labrusca Niagara Rosada	PI 288688
V. labrusca Vergennes	PI 588128
V. monticola	DVIT 1302
V. monticola	DVIT 1369
V. monticola	DVIT 1576
V. monticola	DVIT 1847
V. monticola	PI 588454
V. monticola	PI 588627
V. mustangensis	DVIT 1134
V. mustangensis	DVIT 1277
V. mustangensis	DVIT 1577
V. mustangensis	DVIT 1844
V. palmata	DVIT 2227
V. palmata	DVIT 2228
V. palmata	PI 588155
V. palmata	PI 588201
V. palmata Cache 8	PI 588233
V. piasezkii[y]	PI 588465
V. riparia	PI 588054
V. riparia	PI 588259
V. riparia	PI 588262
V. riparia	PI 588271
V. riparia	PI 588273
V. riparia	PI 588435
V. riparia	PI 588436
V. riparia	PI 588438
V. riparia	PI 588439
V. riparia	PI 588440
V. riparia	PI 588458
V. riparia	PI 588459
V. riparia	PI 588483
V. riparia	PI 588565
V. riparia	PI 588580
V. riparia	PI 588586
V. riparia	PI 588710
V. riparia Gloire	PI 588214
V. riparia Quebec	PI 588345
V. riparia Zumbrunner	PI 594344
V. rotundifolia Thomas	DVIT 150
V. rotundifolia	DVIT 1145
V. rotundifolia Albermarle	DVIT 1738
V. rotundifolia Stucky	DVIT 1743
V. rupestris	PI 588146
V. rupestris	PI 588147
V. rupestris	PI 588160
V. rupestris	PI 588223
V. rupestris	PI 588224
V. shuttleworthii	DVIT 1713
V. shuttleworthii	DVIT 1714
V. shuttleworthii	DVIT 2249
V. shuttleworthii Kissimmee	DVIT 2387
V. vinifera Cabernet Sauvignon[y]	POOL V21-2-10
V. vinifera Chardonnay Clone 95[y]	POOL RS3-18-20
V. vinifera Feteasca Alba[y]	PI 348888
V. vinifera Merlot[y]	POOL RS1-36-31
V. vinifera Morio Muscat[y]	PI 588425
V. vinifera Pinot Noir[y]	POOL RS1-38-31
V. vinifera Riesling[y]	POOL V21-3-4
V. vinifera Rkaziteli[y]	PI 588177
V. vinifera Saperavi[y]	PI 588691
V. vinifera Tarnau[y]	PI 313922
V. vinifera Thompson Seedless mutant[y]	PI 588236
V. vinifera Viognier[y]	PI 594339
V. vinifera West Freesia[y]	PI 588708
V. vulpina	PI 588133
V. vulpina	PI 588142
V. vulpina	PI 588185
V. vulpina	PI 588356
V. vulpina	PI 588679
V. ×*andersonii*[y]	PI 588392
V. ×*champinii*	PI 588371
V. ×*champinii* Salt Creek	PI 588123
V. ×*doaniana*	PI 588149
V. ×*novae-angliae*	PI 588257
V. yenshanensis[y]	PI 588421

[z]PI numbers are those of USDA–ARS Plant Genetic Resources Unit, Cornell Univ., Geneva, N.Y. DVIT numbers are those of the USDA–ARS National Germplasm Repository, Univ. of California, Davis. POOL numbers are designations for plants in the collection of Robert Pool, Dept. of Horticultural Sciences, Cornell Univ.

[y]An accession of European or Asian origin; all other accessions are native to North America.

not be conserved across all the species in the PGRU and NGR collections. Although Thomas and Scott (1993) and Bowers et al. (1996) successfully fingerprinted all sexually, independently derived *V. vinifera* cultivars and successfully amplified SSR fragments in some other species and interspecific hybrids as well, the primers still must be tested empirically on each species of interest. Not all primers developed for one species will necessarily prime amplifications in all closely related species (Röder et al., 1995).

Second, the known SSR loci may not be polymorphic enough to enable all genotypically unique accessions within the PGRU and NGR collections to be unambiguously distinguished. Even if PCR amplification is successful within all of the species, not all accessions within every species necessarily will possess distinguishing SSR fingerprints. Previous grape workers (Bowers et al., 1996; Thomas and Scott, 1993; Thomas et al., 1994), however, did find sufficient polymorphism to distinguish all genetically distinct accessions in *V. vinifera* (except somatic mutations or sports).

In this study, we tested whether SSR primers, developed for DNA fingerprinting *V. vinifera*, would amplify DNA fragments across a wide range of *Vitis* germplasm selected from the PGRU and NGR collections. In addition, we investigated if the polymorphism found would be great enough that distinct genotypes in those *Vitis* collections would have a high likelihood of displaying unique SSR profiles.

Material and Methods

Plant material. An array of 110 plants (Table 1) was selected for SSR analysis. Five cultivars of *V. vinifera*—'Cabernet Sauvignon', 'Chardonnay', 'Merlot', 'Pinot Noir', and 'Riesling'—were used as standards to verify that the correct DNA fragments were being amplified and to establish size relationships between DNA fragments found in our study and those found by previous workers (Bowers et al., 1996; Thomas et al., 1994). The other 105 plants were selected to represent all *Vitis* taxa (16 species and 3 named hybrids) native to North America north of Mexico (Moore, 1998), as well as five additional Old World grape species and one hybrid. This array includes all grape species having more than two accessions in the PGRU or NGR collections except *V. jacquemontii* R.N. Parker. No other hybrids were included in this study, since most have the analyzed species in their pedigrees. Nomenclature of North American *Vitis* species is based on the taxonomic classification of Moore (1998), while that for Old World species follows Galet's (1988, 1990) classification.

DNA extraction. Leaf tissue of each accession was taken from single individuals: greenhouse cuttings from plants in the PGRU collection (harvested January to March 1996), vines from either the PGRU collection or that of Robert Pool, Dept. of Horticultural Sciences, Cornell Univ. (harvested July to August 1996), or plants from the NGR vineyard, whose leaves were sent to Geneva by overnight mail in sealed plastic bags containing damp paper towels (harvested June to July 1996).

DNA was extracted using the following novel microprep protocol we developed for use with small quantities of tissues rich in polysaccharides, polyphenolics, and other PCR-inhibiting compounds, because in our hands other protocols tested (Bowers et al., 1993; Dellaporta et al., 1983; Doyle and Doyle, 1991; Lodhi et al., 1994; Ren at al., 1995; Steenkamp et al., 1992; Thomas et al., 1993) did not yield DNA of sufficient purity and longevity. The following protocol gave adequate quality (260/280 ratio >1.6) and quantity (>20 µg/100 mg leaf tissue) of DNA from plants of the genera *Brassica*, *Cyclamen*, *Euphorbia*, *Hyacinthus*, *Laurus*, *Malus*, *Narcissus*, *Origanum*, *Pelargonium*, *Picea*, *Primula*, *Prunus*, *Rosmarinus*, and *Vitis*. DNA obtained from this protocol has been used in successful PCR reactions after >2 years storage at –20 °C.

About 100 mg of young, expanding leaf tissue was placed in a chilled mortar (–20 °C), covered with liquid N_2, and ground to a fine powder with a chilled (–20 °C) pestle. The powder was quickly scraped together and transferred to a 1.5-mL microfuge tube held on ice. Then 600 µL of extraction buffer [250 mM Tris-HCl (pH 8.0), 250 mM NaCl, 50 mM EDTA, 50 mM diethyldithiocarbamic acid (sodium salt), 100 mM sodium ascorbate, 4.0% PVP-40 (w/v), 1.5% SDS (w/v), and 1.0% β-mercaptoethanol (v/v) (added just before use)] was added and mixed thoroughly.

The tissue–buffer mixture was held on ice until all samples were ground. Samples were incubated in a water bath at 37 °C for 30 min, with gentle inversion once every 10 min. Tubes were removed from the water bath and 1.0 volume of 4 °C 24 chloroform : 1 isoamyl alcohol (v/v) was added, the tube was gently mixed for 5 min to form a thorough emulsion, the sample was microfuged at 13,000 rpm for 15 min at 4 °C, and the aqueous (top) layer was transferred to a new tube and the organic layer discarded. A 0.7 v of 5.0 M NaCl was added to the aqueous layer and mixed gently but thoroughly. This mixture was microfuged at 13,000 rpm for 30 min at 4 °C, and the supernatant was removed to a new tube. DNA was precipitated by adding 0.66 v of isopropanol (–20 °C), mixing gently but completely, and placing the sample in –20 °C for 30 min. The sample was microfuged at 13,000 rpm for 15 min at 4 °C, and the supernatant was decanted, leaving the DNA pellet. The pellet was rinsed with washing buffer (76% ethanol/0.01 M ammonium acetate at –20 °C) for 5 min and the washing buffer was discarded. The pellet was washed briefly with 70% ethanol (–20 °C), the alcohol was decanted, and the pellet was allowed to air dry at room temperature. The pellet was suspended in 300 µL of TE, 15 µg of heat-treated RNAase A (1 $\mu g \cdot \mu L^{-1}$) was added, and the mixture incubated in a water bath at 65 °C for 10 min. Samples were removed from the water bath and 0.4 v (126 µL) of 7.5 M ammonium acetate was added to precipitate proteins (including the RNAase A). Samples were allowed to stand 30 min at 4 °C, microfuged at 13,000 rpm for 15 min at 4 °C, and supernatant containing DNA was carefully removed to a new tube with a micropipette. DNA was precipitated using 2 v of 100% ethanol (–20 °C) and the tubes were placed at –20 °C for 30 min. Samples were then microfuged at 13,000 rpm for 15 min at 4 °C. The alcohol was decanted and the pellet was treated with washing buffer (–20 °C) for 5 min. The washing buffer was discarded, the pellet was washed briefly with 70% ethanol (–20 °C), the alcohol was discarded, and the pellet was allowed to air dry at room temperature. It was then dissolved in 200 µL of TE. DNA concentration was determined using a RNA–DNA calculator (GeneQuant II; Pharmacia Biotech Inc., Piscataway, N.J.) or a spectrophotometer (Spectronic 2000; Bausch and Lomb, Rochester, N.Y.). If the DNA was of satisfactory purity at this stage, the following steps were omitted. If additional purification was required, 0.10 v of 4.0 M NaCl was added, the solution was mixed gently and placed at 4 °C for 10 min. The sample was then microfuged at 13,000 rpm for 15 min at 4 °C, and the supernatant containing DNA was removed to a new tube. Two volumes of 100% ethanol (–20 °C) were added and tubes were placed at –20 °C for 30 min, then microfuged at 13,000 rpm for 15 min at 4 °C. The DNA pellet was rinsed with washing buffer (–20 °C) for 5 min, and the buffer was decanted. The pellet was washed briefly with 70% ethanol, the alcohol was discarded, and the pellet was allowed to air dry at room temperature. The DNA was dissolved in 200 µL of TE. In 12 h, one person can complete the entire DNA extraction procedure (including quantification) for 24 samples.

PCR amplification. Primer pairs (Table 2) were synthesized from published sequences and fluorescently labeled at the DNA Sequencing Facility, Dept. of Entomology, Cornell Univ., or at the New York

Table 2. SSR primer designations, sequences, and references.

Primer[z]	Sequence (5' to 3')	Reference
VVS2A-6-FAM	CAG CCC GTA AAT GTA TCC AT	Thomas and Scott (1993)
VVS2B	AAA TTC AAA ATT CTA ATT CAA CTG G	
VVS4A	CCA TCA GTG ATA AAA CCT AAT GCC	Thomas and Scott (1993)
VVS4B-HEX	CCC ACC TTG CCC TTA GAT GTT A	
VVMD6A-TET	ATC TCT AAC CCT AAA ACC AT	Bowers et al. (1996)
VVMD6B	CTG TGC TAA GAC GAA GAA GA	
VVMD7A-HEX	AGA GTT GCG GAG AAC AGG AT	Bowers et al. (1996)
VVMD7B	CGA ACC TTC ACA CGC TTG AT	
VVMD8A-6-FAM	TAA CAA ACA AGA AGA GGA AT	Bowers et al. (1996)
VVMD8B	AGC ACA TCC ACA ACA TAA TG	

[z]6-FAM, TET, and HEX (Perkin-Elmer Applied Biosystems, Inc.) represent fluorescent 5' end-labels.

State Center for Advanced Technology, Advanced Chemistry and Peptide/DNA Synthesis Facility, Cornell Univ., on a nucleic acids synthesizer (model 392; Perkin Elmer Applied Biosystems, Inc., Foster City, Calif.). During synthesis, one member of each primer pair was fluorescently labeled with a Perkin Elmer fluorphore, 6-FAM (blue), TET (green), or HEX (yellow).

Two different multiplexed PCR reactions were carried out, depending on the primers used. The first reaction had a total volume of 25 μL and consisted of 1× ThermoPol reaction buffer (New England Biolabs, Beverly, Mass.); 2.5 mM $MgSO_4$, 425 μM each of dATP, dCTP, dGTP, and dTTP; 5 pmol of each primer pair (forward and reverse) of both primers VVS2 and VVS4; 37.5 ng of template DNA; and 0.25 unit (U) of a high fidelity, thermophilic DNA polymerase with integral 3' to 5' exonuclease activity (Deep Vent; New England Biolabs). The PCR reaction was held at 94 °C for 6 min, then run for 45 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 100 s. The second reaction had a total volume of 25 μL and contained 1× ThermoPol reaction buffer; 2.25 mM $MgSO_4$; 425 μM each of dATP, dCTP, dGTP, and dTTP; 4 pmol of each primer pair (forward and reverse) of VVMD6; 6 pmol of each primer pair of VVMD7; 120 pmol of each primer pair of VVMD8; 37.5 ng of template DNA; and 0.25 U of DNA polymerase. The PCR reaction was held at 94 °C for 6 min, then run for 45 cycles of 94 °C for 1 min, 50 °C for 100 s, and 72 °C for 2 min. Deep Vent DNA polymerase was used for all PCR reactions because it generated fewer PCR artifacts—stutter bands (Perlin et al., 1995; Smeets et al., 1989) and +A bands (Clark, 1988; Mayrand et al., 1992). All PCR amplifications were carried out in a thermocycler (model 9600; Perkin-Elmer).

Gel electrophoresis and SSR fragment sizing. To detect DNA fragments, 1 μL of the 25-μL PCR reaction was mixed thoroughly with 0.5 μL of internal lane standard (GENESCAN-350 TAMRA; Perkin Elmer), 0.25 μL loading dye, and 1.25 μL formamide. This mixture was heated at 90 °C for 4 min to denature. After denaturation, samples were placed immediately on ice and after cooling loaded onto 36-well, 24-cm sequencing gels (6% acrylamide, 8.3 M urea, and 1× Tris EDTA borate) prepared according to the manufacturer's specifications. Gels were run for 12 h on a automated DNA sequencer (model 377 or 373; Perkin Elmer) at the Applied Genetic Analysis Laboratory of the USDA–ARS Plant Genetic Resources Conservation Unit, Griffin, Ga., or in the Geneva DNA Sequencing Facility, Dept. of Entomology, Cornell Univ., respectively. Fluorescently labeled amplified fragments were detected and initially sized using GENESCAN 672 software (Perkin Elmer).

With the electrophoretic conditions specified above, the GENESCAN software assigned fractional bp sizes to DNA fragments. The program BINNING, written by Lamboy in Microsoft Fortran Powerstation for MS-DOS and Windows (Microsoft Inc., Redmond, Wash.), was used to determine which fractional-sized fragments represented the same integer-sized fragments. Two or more replicate PCR amplifications and electrophoresis runs were carried out on most samples to ensure reproducibility of fragment sizing. This method of scoring ensured consistent assignment of fragment sizes across different gels and is analogous to the binning that is done with variable number of tandem repeat fragments (Budowle et al., 1991; Weir, 1992).

SSR data analysis. Data compilation, comparison of DNA fingerprints across accessions, and computation of percent heterozygosity, gene diversity (Weir, 1990) [also referred to as polymorphic information content (PIC) (see, Röder et al., 1995)], gene and genotype frequencies, and discrimination power (Jones, 1972; Kloosterman et al., 1993) were carried out using the computer program SSRS, written by Lamboy using Microsoft Fortran Powerstation for MS-DOS and Windows. Here, the discrimination power is the probability that two grape accessions can be distinguished by their SSR profiles. It is computed as one minus the probability that the SSR profiles will be identical (Jones, 1972). The method assumes that the plants from which SSR data are collected represent a random sample of the population about which inferences are made.

Since Thomas et al. (1994) showed Mendelian inheritance of SSR fragments produced by primer sets VVS2 and VVS4, and we have proven it for primer sets VVMD6 and VVMD7 using a mapping population of 'Illinois 547-1' x 'Horizon' (W.F. Lamboy, C.G. Alpha, M. Dalbo, B.I. Reisch, and N.F. Weeden, unpublished data), we freely refer to SSR fragments as alleles and their chromosomal locations as loci. This usage was also followed by Bowers et al. (1996).

Results

All five primer pairs produced SSR fragments for all accessions (Table 3). We found no null loci, although these were occasionally found by Thomas et al. (1994). A complete compilation of the alleles displayed by all accessions can be accessed at http://www.ars-grin.gov/ars/NoAtlantic/Geneva/vitis_ssr.html. The data is also available from W.F. Lamboy in hard copy or word processing format.

For the five *V. vinifera* standard cultivars, amplification by primers VVS2, VVS4, and VVMD7 produced fragments with sizes identical to those reported by Thomas et al. (1994) or Bowers et al. (1996) (Table 4). All of the fragments amplified by primer VVMD6 were consistently and reproducibly 5 bp shorter than found by Bowers et al. (1996). This size difference is not surprising since we used fluorescent detection and internal lane standards for sizing fragments, whereas Bowers et al. (1996) used silver staining and sequencing reactions. With primer VVMD8, all but one of our fragments were 4 bp shorter than the corresponding fragments of

Table 3. Number of SSR fragments observed and fragment sizes in base pairs (bp) for each of the five SSR primer pairs used to fingerprint 110 *Vitis* germplasm accessions.

	SSR fragments observed	
Primer	No.	Sizes (bp)
VVS2	25	121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 159, 161, 163, 167, 173, 175, 191
VVS4	20	155, 159, 163, 165, 167, 168, 169, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197
VVMD6	16	190, 195, 200, 203, 204, 206, 207, 208, 209, 211, 213, 215, 217, 219, 225, 227
VVMD7	23	217, 229, 231, 233, 234, 235, 237, 239, 241, 243, 244, 245, 247, 249, 250, 251, 253, 255, 257, 259, 261, 263, 265
VVMD8	38	131, 135, 137, 139, 141, 143, 145, 147, 149, 155, 157, 159, 161, 163, 164, 166, 167, 168, 169, 171, 172, 173, 174, 175, 176, 177, 178, 179, 181, 183, 185, 187, 190, 191, 193, 195, 197, 199

Bowers et al. (1996). The exception is the 157-bp fragment (Bowers et al., 1996) in 'Cabernet Sauvignon' and 'Merlot', which is 155 bp in our study. We have not yet determined the cause of this anomaly, but the expected fragment size of 153 is located in a 3 bp break (151–153) in our observed series of odd-numbered size fragments, 135–163 bp (Table 3). Except for this anomaly, conversion from one lab's fragment sizes to another's was straightforward.

Allowing for constant size differences between our results and those of Bowers et al. (1996), all of the alleles they observed at VVMD6, VVMD7, and VVMD8, except 157, were also seen by us (Table 3) . Similarly, all of the fragments for primers VVS2 and VVS4 listed by Thomas et al. (1994) in their Table 1 were detected in this study (Table 3), with no adjustment needed for size differences between labs. Although it would be cumbersome to list all alleles and their frequencies, the most common alleles (having frequencies of ≥0.05) are shown in Table 5.

The minimum number of different alleles found at a locus was 16, while the maximum was 38 (Table 3), with an average of 24.4 per locus. Plants showing only a single amplified fragment were assumed to be homozygous for that fragment for purposes of computing the percent heterozygosity, gene diversity, and gene and genotype frequencies, rather than assuming that they were heterozygous for a null allele. If our assumption is incorrect, and null alleles are present in any of the apparently homozygous plants, heterozygosity and gene diversity values will be underestimates.

Heterozygosity values ranged from 0.464 to 0.818 with an average of 0.625, and gene diversity (PIC) values varied from 0.875 to 0.955 with an average of 0.911 (Table 6). The lowest discrimination power (probability that two randomly selected accessions possess different SSR profiles) at a locus (VVMD6) was 0.947; the highest (at VVMD8) was 0.987 (Table 6). The discrimination power for all loci combined was effectively 1.0.

The SSR profiles combined over the five loci were compared for all 5995 different pairs of accessions to determine if any plants were genetically identical. Of these pairs, 5395 (90%) were different at all five loci, 518 (8.6%) differed at four loci, 60 (1.0%) differed at three loci, 21 (0.35%) differed at two loci, and one pair of plants, PI 588356 (*V. vulpina* L.) and PI 588199 (*V. cinerea* (Engelm. in Gray) Engelm. ex Millardet), did not differ at any locus, i.e., their genetic fingerprints were identical. No plants differed at just one locus. Thus, all 110 accessions had unique fingerprints except PI 588356 and PI 588199.

Discussion

Five primer pairs developed for fingerprinting *V. vinifera* cultivars amplified SSR fragments in 19 native North American *Vitis* species and hybrids and six Old World *Vitis* taxa studied. All but one pair of plants were unambiguously distinguished from one another by their SSR profiles. This result is concordant with previous SSR analyses of grape, in which all *V. vinifera* genotypes were distinguished, except those that were known to be or suspected of being genetically identical (Botta et al., 1995; Thomas and Scott, 1993) or were somatic mutants (Bowers et al., 1996).

The level of polymorphism detected at the five loci was high. Sixteen different alleles were resolved at the least polymorphic locus (VVMD6), while 38 were identified at the most polymorphic locus (VVMD8). In their studies of cultivars of *V. vinifera*, Thomas and Scott (1993) found a minimum of 4 and a maximum of 13 alleles at a locus, while Bowers et al. (1996) found a minimum of 6 and a maximum of 11 alleles. Since the present study used 25 species and hybrids, one would expect to detect a greater number of different alleles per locus. Saghai Maroof et al. (1994) reported finding 28 and 37 different alleles at two loci in their study of an array of 207 wild and cultivated barley (*Hordeum vulgare* L.) accessions, which were the largest numbers reported up until that time. Our study uncovered a comparable number of different alleles among 110 accessions in a taxonomically much broader array of germplasm.

Only 1 (0.8%) of the 122 alleles detected in this study had a

Table 4. Comparison of SSR fragment sizes obtained in this study to fragment sizes obtained by previous workers for standard grape cultivars.

Primer	VVS2	VVS4	VVMD6	VVMD7	VVMD8
Cabernet Sauvignon	139, 151[z]	168, 175[z]	211, 212[y]	239, 239[y]	143, 157[y]
	139, 151[x]	168, 175[x]	206, 207[x]	239, 239[x]	139, 155[x]
Chardonnay	137, 143[z]	168, 173[z]	205, 214[y]	239, 243[y]	141, 147[y]
	137, 143[x]	168, 173[x]	200, 209[x]	239, 243[x]	137, 143[x]
Merlot	139, 151[z]	168, 175[z]	205, 212[y]	239, 247[y]	143, 157[y]
	139, 151[x]	168, 175[x]	200, 207[x]	239, 247[x]	139, 155[x]
Pinot Noir	137, 151[z]	168, 173[z]	205, 205[y]	239, 243[y]	141, 143[y]
	137, 151[x]	168, 173[x]	200, 200[x]	239, 243[x]	137, 139[x]
Riesling	143, 151[z]	168, 168[z]	211, 214[y]	249, 257[y]	143, 147[y]
	143, 151[x]	168, 168[x]	206, 209[x]	249, 257[x]	139, 143[x]

[z]Fragment sizes reported by Thomas et al. (1994).
[y]Fragment sizes reported by Bowers et al. (1996).
[x]Fragment sizes from this study.

Table 5. SSR fragments[z] detected in 110 grape accessions and having relative frequency (f) of ≥0.05.

VVS2		VVS4		VVMD6		VVMD7		VVMD8	
bp	f	bp	f	bp	f	bp	f	bp	f
125	0.068	168	0.086	200	0.095	231	0.082	149	0.055
133	0.100	173	0.073	204	0.123	233	0.055	157	0.100
137	0.105	175	0.218	206	0.077	235	0.105	159	0.068
139	0.077	177	0.159	207	0.118	239	0.155	166	0.064
141	0.082	179	0.091	209	0.195	241	0.082	179	0.064
143	0.109	181	0.055	213	0.055	251	0.155		
145	0.095	185	0.127	215	0.191				
151	0.055								
Others[y]	0.309		0.191		0.146		0.366		0.649

[z]SSR fragments are identified by their length in base pairs (bp).
[y]Cumulative frequency of all fragments having relative frequency <0.05 is listed as others.

frequency of >0.20 (Table 7). This compares to 8 of 30 alleles (26.7%) (Bowers et al., 1996) and 2 of 26 alleles (7.7%) (Thomas and Scott, 1993) (Fig. 3 A and B) that were this common in previous studies. In addition, 89 alleles (73.0%) had an allelic frequency of <0.05, while in previous studies 17 (65.4%) (Thomas and Scott, 1993) and 13 (43.3%) (Bowers et al., 1996) such alleles were found. Thus, rare alleles were more common and common alleles were rarer in this study than in the previous *Vitis* studies, which sampled more taxonomically restricted sets of accessions.

Thomas and Scott (1993) found levels of SSR heterozygosity in cultivated grapes of 0.69 to 0.88, with a mean of 0.778, while Bowers et al. (1996) reported values of 0.75 and 0.93 (mean = 0.86) for these quantities. Heterozygosity levels in this study ranged from 0.464 to 0.818 with a mean of 0.625. If the 13 *V. vinifera* cultivars are excluded, heterozygosity values in this study are even lower, with a minimum of 0.414, a maximum of 0.814, and a mean value of 0.600. Whether cultivated grapes typically have higher levels of heterozygosity than wild grapes is unknown. We hypothesize that they do, however, since heterozygosity generally confers hybrid vigor (Grant, 1975), resulting in such desirable characteristics as higher fruit production, larger plant size, and more rapid root growth.

One of the goals of this study was to obtain an estimate of the likelihood that all sexually, independently derived accessions in the PGRU and NGR germplasm collections would display unique fingerprints. Such an estimate is provided by the discrimination power (Jones, 1972; Kloosterman et al., 1993), defined as the probability that two randomly selected accessions will be distinguishable by their allelic profiles. Based on the data from all five loci, the discrimination power in this study is computed to be $1.0000 - (0.208 \times 10^{-7})$, effectively 1.0. Inversely, there are therefore about two chances in one hundred million that two accessions, selected randomly from a population possessing the allelic frequencies observed in this study, will have identical genotypes.

Some plants in the PGRU and NGR collections are more closely related than the plants so far analyzed. For example, some accessions in the collections are seedlings from the same maternal parent, some are sports of known cultivars, and some have identical parents in their pedigrees. For these related plants, the above discrimination power is an overestimate. Nevertheless, earlier successes (Bowers et al., 1996; Thomas et al., 1994) in fingerprinting >150 *V. vinifera* clones, many of which are closely related, combined with the high discrimination power observed in this study, provides strong evidence that the SSR loci analyzed will be adequate for DNA fingerprinting all sexually, independently derived accessions in the PGRU and NGR genetic resources grape collections.

Initially, one of the primary uses of SSRs in the USDA–ARS *Vitis* collections will be to identify duplicate accessions and those that are very closely related. In this study, only two plants—PI 588356 (*V. vulpina*) and PI 588199 (*V. cinerea*) (Table 1)—were genetically identical at all five loci. The probability that two unrelated accessions both would have this specific genotype is 5.5×10^{-8}. (This value is obtained by multiplying the estimated genotype frequencies.) Independent data from >200 random amplified polymorphic DNA (RAPD) bands amplified from the same DNA samples shows that the two accessions possess the same RAPD profiles (unpublished data). From these results, we conclude that PI 588356 and PI 588199 indeed possess the same genotype.

A review of our passport information for these two accessions suggests how the two plants came to be classified as belonging to two different species. Passport information for PI 588356 showed that the

Table 6. Heterozygosity, gene diversity, probability of two fingerprints matching by chance, and discrimination power at each locus and for all five loci combined[z].

Locus	Heterozygosity	Gene diversity or polymorphic information content (PIC)	Probability of matching fingerprints	Discrimination power
VVS2	0.673	0.929	0.0210	0.979
VVS4	0.627	0.883	0.0374	0.963
VVMD6	0.464	0.875	0.0534	0.947
VVMD7	0.545	0.915	0.0370	0.963
VVMD8	0.818	0.955	0.0134	0.987
Mean	0.625	0.911	---	---
All loci	---	---	0.208×10^{-7}	1.000

[z]Probability of a match at all five loci combined is the product of the five individual locus probabilities. Discrimination power is one minus the probability of a matching fingerprint. All values were rounded to three significant digits after computation.

Table 7. The number of alleles belonging in various allelic frequency classes for the five loci studied.

Allelic frequency class	Alleles within a class (no.)
<0.050	89
0.050–0.099	19
0.100–0.149	8
0.150–0.199	5
>0.200	1

original identity of the plant had been lost, and a provisional species identification had been made after planting. In Summer 1996, we reidentified both grapevines using taxonomic keys of Fernald (1950) and Moore (1998), and both plants were identified as *V. cinerea*, which is distinguishable from *V. vulpina* by the presence of hairy branchlets that are distinctly angled (in cross section), while *V. vulpina* has smooth branchlets that are circular (in cross section). Both plants were obtained originally from the same breeder, so it was not surprising to discover that they have the same genotype. An error must have occurred during propagation, planting, or documentation. The discovery of these genotypically identical plants confirms the use of SSRs for fingerprinting individual plants and for establishing genetic identity of clonal grape accessions.

Twenty-one pairs of plants differed at two loci. Of these, 14 pairs were combinations of the *V. cinerea* accessions—PI 588199, PI 588220, PI 588222, PI 588460, PI 588688, and PI 588356 (mentioned above). Other pairs of accessions that differed at only two loci were *V. amurensis* Rupr. (PI 588382) from China and *V. vulpina* (PI 488142) from New Jersey; two accessions of *V. girdiana* Munson (DVIT 1379 and DVIT 1380); *V. mustangensis* Buckl. (DVIT 1844) and *V. girdiana* (DVIT 1379); two *V. labrusca* L. accessions (PI 588165 and PI 588583); *V. rotundifolia* Michx. (DVIT 1145) and the hybrid *V.* ×*champinii* Planch. (PI 588123); *V. labrusca* (PI 588165) and *V.* ×*champinii* (PI 588371); and finally, the two *V. vinifera* cultivars ('Cabernet Sauvignon' and 'Merlot'). Of the 21 pairs of accessions that differed at only two loci, 17 (81%) belonged to the same species. Thus, only 4 out of 5995 pairs of accessions had different SSR profiles at 2 loci or fewer and belonged to different species. This provides further evidence that the likelihood of two unrelated grape accessions sharing by chance the same SSR alleles at all five loci is remote.

Based on the success of the present study, we plan to use SSRs to fingerprint additional *Vitis* accessions in the PGRU and NGR germplasm collections. In fact, fingerprinting of the PGRU core subset, a set of accessions selected to represent the genetic diversity in the entire PGRU grape collection, has already begun. We are also verifying the identities and pedigrees of several dozen commercially important cold-hardy grape clones using SSRs. Because they are highly polymorphic, codominant markers, with loci that are likely to be conserved across a range of related species, we strongly recommend using SSRs for fingerprinting not only grape germplasm but also other clonal genetic resource collections as well.

Literature Cited

Alleweldt, G., P. Spiegel-Roy, and B. Reisch. 1990. Grapes (*Vitis*), p. 289–327. In: J.N. Moore and J.R. Ballington (eds.). Genetic resources of temperate fruit and nut crops. I. Intl. Soc. Hort. Sci., Wageningen, The Netherlands.

Botta, R., N.S. Scott, I. Eynard, and M.R. Thomas. 1995. Evaluation of microsatellite sequence-tagged site markers for characterizing *Vitis vinifera* cultivars. Vitis 34:99–102.

Bowers, J.E., E.B. Bandman, and C.P. Meredith. 1993. DNA fingerprint characterization of some wine grape cultivars. Amer. J. Enol. Viticult. 44:266–274.

Bowers, J.E., G.S. Dangl, R. Vignani, and C.P. Meredith. 1996. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 39:628–633.

Budowle, B., A.M. Giusti, J.S. Wayne, F.S. Baechtel, R.M. Fourney, D.E. Adams, L.A. Presley, H.A. Deadman, and K.L. Monson. 1991. Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data for VNTR loci for use in forensic comparisons. Amer. J. Hum. Genet. 48:841–855.

Clark, J.M. 1988. Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerase. Nucl. Acids Res. 16:9677–9686.

Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: version II. Plant Mol. Biol. Rpt. 1:19–21.

Doyle, J.J. and J.L. Doyle. 1991. Isolation of plant DNA from fresh tissue. Focus 12:13–15.

Fernald, M.L. 1950. Gray's manual of botany. D. Van Nostrand, New York.

Galet, P. 1979. A practical ampelography. Comstock Publ. Assoc., Ithaca, N.Y.

Galet, P. 1988. Cépages et Vignobles de France. vol. 1. Les Vignes Américaines. Charlés Dehan, Montpellier, France.

Galet, P. 1990. Cépages et Vignobles de France. vol. 2. L'Ampelographie Française. Charlés Dehan, Montpellier, France.

Grant, V. 1975. Genetics of flowering plants. Columbia Univ. Press, New York.

Jones, D.A. 1972. Blood samples: Probability of discrimination. J. Forens. Sci. Soc. 12:355–359.

Kloosterman, A.D., B. Budowle, and P. Daselaar. 1993. PCR-amplification and detection of the human D1S80 VNTR locus. Intl. J. Leg. Med. 105:257–264.

Kresovich, S. and J.R. McFerson. 1992. Assessment and management of plant genetic diversity: Considerations of intra- and interspecific variation. Field Crops Res. 29:185–204.

Lodhi, M.A., G.-N. Ye, N.F. Weeden, and B.I. Reisch. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Rpt. 12:6–13.

Mayrand, P.E., K.P. Corcoran, J.S. Ziegle, J.M. Robertson, L.B. Hoff, and M.N. Kronick. 1992. The use of fluorescence detection and internal lane standards to size PCR products automatically. Appl. Theor. Electroph. 3:1–11.

McFerson, J.R., W.F. Lamboy, and S. Kresovich. 1996. Assessing user perceptions of genetic resources collections in crucifer crops. Crop. Sci. 36:831–838.

Moore, M. 1998. Vitaceae. In: The flora of North America. vol. 5. Oxford University Press, New York.

Peeters, J.P. and N.W. Galwey. 1988. Germplasm collections and breeding needs in Europe. Econ. Bot. 42:503–521.

Perlin, M.W., G. Lancia, and S.-K. Ng. 1995. Toward fully automated genotyping: Genotyping microsatellite markers by deconvolution. Amer. J. Hum. Genet. 57:1199–1210.

Ren, J., J.R. McFerson, R. Li, S. Kresovich, and W.F. Lamboy. 1995. Identities and relationships among Chinese vegetable brassicas as determined by random amplified polymorphic DNA markers. J. Amer. Soc. Hort. Sci. 120:548–555.

Röder, M.S., J. Plaschke, S.U. König, A. Börner, M.E. Sorrells, S.D. Tanksley, and M.W. Ganal. 1995. Abundance, variability, and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246:327–333.

Saghai Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, and R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. Proc. Natl. Acad. Sci. USA 91:5466–5470.

Smeets, H.J.M., H.G. Brunner, H. Rogers, and B. Wieringa. 1989. Use of variable simple sequence motifs as genetic markers: Application to study of myotonic dystrophy. Hum. Genet. 83:245–251.

Steenkamp, J., I. Wiid, A. Lourens, and P. Van Helden. 1992. Improved method for DNA extraction from *Vitis vinifera*. Amer. J. Enol. Viticult. 45:102–106.

Thomas, M.R. and N.S. Scott. 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86:985–990.

Thomas, M.R., P. Cain, and N.S. Scott. 1994. DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. Plant Mol. Biol. 25:939–949.

Thomas, M.R., S. Matsumoto, P. Cain, and N.S. Scott. 1993. Repetitive DNA of grapevine: Classes present and sequences suitable for cultivar identification. Theor. Appl. Genet. 86:173–180.

Weir, B.S. 1990. Genetic data analysis. Sinauer Assoc., Sunderland, Mass.

Weir, B.S. 1992. Independence of VNTR alleles defined as fixed bins. Genetics 130:873–887.