Investigations into the Origin of 'Norton' Grape using SSR Markers

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‘Norton’ produces excellent wine in some regions where Vitis vinifera is difficult to grow. The high-quality and pest resistance of ‘Norton’ make it attractive to generate hybrids of similar parentage, producing cultivars with traits distinct from ‘Norton’ but with similar adaptation. ‘Norton’ is frequently described as V. aestivalis, but was initially declared a hybrid between an American grape (‘Bland’) and V. vinifera (‘Miller’s Burgundy’, a synonym for ‘Pinot Meunier’). To try to identify the parents of ‘Norton’, simple sequence repeat (SSR) markers were compared across V. vinifera cultivars and accessions derived from American species. The precise parentage could not be identified using available data. Allele frequencies were compared among 181 Euvitis of North American origin and 354 V. vinifera cultivars for which there were data at 13 loci. At least one allele found in ‘Norton’ at all 13 loci was also present among the vinifera cultivars, while at 6 loci the other allele in ‘Norton’ did not occur among the vinifera cultivars, suggesting these alleles may derive from a non-vinifera parent. Allelic frequency distributions for different Vitis series indicated that the putative non-vinifera ‘Norton’ alleles were common within the aestivalis. These data are consistent with ‘Norton’ being a hybrid with ancestry including V. aestivalis and V. vinifera. ‘Norton’ alleles for locus VVMD36 are rare and may offer the best opportunity for identifying ‘Norton’ parents. Interestingly, ‘Enfariné noir’, a vinifera cultivar which has similarities in synonymy, morphology, and origin with ‘Pinot Meunier’, shares the rare as well as most common alleles with the presumed ‘Norton’ vinifera parent.

The ‘Norton’ grape is grown in many US wine growing regions where Vitis vinifera L. production requires extensive pesticide use, especially in the humid southern and midwestern US (Ambers and Ambers, 2004). ‘Norton’ is noteworthy for being quite disease and pest resistant, therefore requiring minimal pesticide use, while also producing wines which many tasters report to be similar to quality vinifera wine. Hedrick (1908) reports that ‘Norton’ is more resistant to fungal diseases than other “native grapes” and very resistant to phylloxera (Hedrick, 1908), and ‘Norton’ is specifically mentioned as “only slightly susceptible to black rot, powdery mildew, Botrytis and anthracnose and only moderately susceptible to downy mildew” and therefore suitable for growing with reduced spraying (North Central IPM Center, 2000) and displays tolerance to Pierce’s disease (Kamas et al., 2000). ‘Norton’ has been routinely reported to be a grape of American origin, frequently described as being in the species Vitis aestivalis Michx. (e.g., Hogan et al., 2009; Main and Morris, 2004) or Vitis aestivalis–derived (Hou et al., 2002; Reisch et al., 1993). The first report of the ‘Norton’ grape by William Prince (1830) of the renowned Prince Nursery of Flushing, NY, indicates that the ‘Norton’ was a seedling produced in the Virginia garden of Dr. Norton as a hybrid of the ‘Bland’ grape and likely ‘Miller’s Burgundy’. There is evidence that this description came directly from Dr. Norton himself (Amber and Amber, 2004). An understanding of the parentage is quite useful in that repeated crosses made with the ‘Norton’ parents might produce a range of quality grape cultivars with pest resistance similar to ‘Norton’ but diverse distinctive traits which may be of value to wine producers (Fig. 1.)

Acknowledgment. Thanks to Dr. Wenping Qiu of Missouri State University for providing the leaves of verified ‘Norton’ that were used in this study.

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Fig 1. ‘Norton’ grape from Hedrick, 1908, “Grapes of New York.”
It is now possible to determine relationships between individual cultivars, using the same methods which are used in human paternity testing. These tests are based on simple sequence repeat (SSR) DNA markers, which are also known as microsatellite markers. These repetitive regions are seldom associated with functional genes, permitting them to accumulate mutations at a relatively high rate, and providing useful diversity for distinguishing individuals. Six carefully selected, highly polymorphic SSR markers in grape provide distinctive markers for virtually all tested V. vinifera cultivars (This et al., 2004) and international adoption of this uniform set has provided an extensive database of genotypes from around the world, permitting comparison to distinguishing individuals.

SSR markers are available for many grape genotypes and confirmation of parent/progeny relationships requires use of such additional SSR markers to reduce the likelihood of erroneous conclusions. Many papers have been published establishing parentage of important V. vinifera cultivars, perhaps most noteworthy that ‘Cabernet Sauvignon’ is likely an offspring of ‘Cabernet Franc’ and ‘Sauvignon Blanc’ (Bowers and Meredith, 1997) and ‘Pinot Noir’ and ‘Gouais Blanc’ are the likely parents of many important varieties including ‘Chardonnay’ (Bowers et al., 1999). We recently published such a study assessing validity of reported parentage for releases from the Cornell grape breeding program (Bautista et al., 2008). Similar methodology was used in this study to explore the likely parents of ‘Norton’.

Materials and Methods

The National Clonal Germplasm Repository (NCGR) in Davis, CA, maintains the national collection of grape genotypes, with more than 3000 Vitis accessions. Included in this collection are 1500 Vitis vinifera, many hundreds of species accessions, and a large number of cultivars of North American origin. During the years 2003–08, SSR markers were run on a substantial portion of the accessions in the NCGR collection, including many North American species and V. vinifera cultivars to provide much of the data used in this study, and SSR marker data from collaborators were acquired for many more accessions. ‘Norton’ material from the Missouri State University collection was included in the evaluation to provide a reference fingerprint of the most studied ‘Norton’ material. Specifically for this study, DNA was newly extracted from this material and 59 additional NCGR accessions that are of North American origin and were likely to be in cultivation when ‘Norton’ was first identified or were construed to be potentially related to ‘Norton’, including many accessions of North American species (data not shown). A variety of sources were used to determine which accessions may have been available to Dr. Norton prior to the origin of the ‘Norton’ grape, with the Prince Nursery Catalogue (1822) being especially useful.

The basic technique for SSR analysis was as follows. Young leaves from near the shoot-tip of rapidly growing grapevines were collected and rapidly dried between blotting paper in sealed, labeled envelopes, which were placed in plastic bags with approximately 20 grams of Drierite (W.A Hammond Drierite Co. Ltd., Xenia, OH). Total DNA was extracted from approximately 20 mg of dried leaf tissue using a DNaseasy Plant Mini Kit (QIAGEN, Valencia, CA) following the manufacturer’s protocol. PCR amplifications were performed on a GeneAmp PCR system model 9700 Thermal Cycler in total 20-µL volume reactions following typical protocols (Dangl et al. 2005). Samples were prepared for capillary electrophoresis by diluting 1.0 µL of amplified product and 0.4 µL of the internal size standard 400HD ROX (ABI) in 12 µL of formamide. Forward primers were labeled with one of three fluorescent dyes. Fragment amplifications were verified on 2% agarose gels. Typically, products from three loci labeled with different fluorescent dyes were multiplexed in PCR and thus also in electrophoresis. Amplified fragments were separated by electrophoresis on an ABI Prism 3100 Genetic Analyzer using 22 cm capillary with 3100 POP-4 as the matrix, and were scored using ABI Genotyper software (Applied Biosystems Inc., Foster City, CA) as described in Dangl et al. (2005).

Most SSR fingerprints were collected using the procedure described above, but some database fingerprints had been scored on gels and allele sizes were rigorously adjusted, using common standard genotype references, to be consistent with data from capillary electrophoresis.

Each sample was analyzed at seven to 13 SSR loci. All analyses included the six internationally adopted reference markers for distinguishing vinifera cultivars (This et al., 2004). In the first study set, the SSR fingerprint of ‘Norton’ for the seven loci VVMD5, VVMD7, VVMD27, VVMD31, VVS2, VrZAG62, and VrZAG79 was compared to the Grape DNA Identification Reference Database maintained by Foundation Plant Services at University of California, Davis (unpublished). This database has more than 1200 unique grape DNA profiles, including V. vinifera, rootstocks and hybrid cultivars. In the second study set, the 13 loci analyzed were VVMD5, VVMD6, VVMD7, VVMD21, VVMD25, VVMD27, VVMD28, VVMD31, VVMD34, VVMD36 (Bowers et al., 1996, 1999), VVS2 (Thomas and Scott, 1993), and VrZAG62, VrZAG79 (Sefc et al., 1999). The frequencies of ‘Norton’ alleles, calculated using Excel Microsatellite Toolkit (Park, 2001) were compared among 181 Euvitis of North American origin and 354 V. vinifera cultivars for which there were data at all 13 SSR loci. Inferences were drawn regarding likely origins of ‘Norton’ based on these allele frequencies.

Genetic distance analyses were conducted on several study subsets, to assess the relatedness of ‘Norton’ to other accessions. Alleles at each locus were used to calculate pairwise genetic distances “proportion of shared alleles” (Bowcock et al., 1994), and dendrograms were constructed using UPGMA (Sneath and Sokal, 1973). Analysis sets with ‘Norton’ included: all 175 Euvitis of North American origin and a subset of 40 diverse V. vinifera cultivars for which there were complete data at all 13 SSR loci, the 175 Euvitis of North American origin alone, and a group of 49 accessions including 6 diverse accessions from each Euvitis series in North America and Europe. In this final analysis, the representatives from each of the six series of Euvitis were selected based on separate dendrograms for each of the series. Six diverse V. vinifera and six hybrids of North American origin were also included.

Results and Discussion

‘Norton’ material from the Missouri State University collection had SSR alleles (Tables 1a and 1b) which were identical to those for the NCGR accessions ‘Cynthiana’ (DVIT0043) verifying synonymy reported based on isozyme profiles (Riesch et al., 1993), and ‘Virginia’ (DVIT0154) reflecting a variation on the reported ‘Norton’ synonyms ‘Virginia Seedling’ and ‘Norton’s Virginia’ (Hedrick, 1908). With co-dominant markers such as SSRs a parent/progeny relationship can be absolutely disproved by any one marker where the two do not share at least one allele. ‘Pinot
Meunier' (synonym for 'Miller's Burgundy'; VIVC, 2009) was clearly not a parent of 'Norton' (Table 1a) as proposed (Prince, 1830). The NCGR accession 'Gold Coin' (DVIT0061) is a T.V. Munson hybrid alleged to have 'Norton' as a parent (Hedrick, 1908), which was consistent with the SSR profile (1b), while the 'Stark Star' (DVIT0145) at the NCGR appears not to be the 'Stark-Star' described by Hedrick (1908) as a likely 'Catawba' by 'Norton' or 'Catawba' by 'Hermann', as it is not a 'Catawba' (Table 1a).

No matches identified possible 'Norton' parents when the SSR fingerprint of 'Norton' using the seven loci VVMD5, VVMD7, VVMD27, VVMD31, VVS2, VrZAG62, and VrZAG79 was compared to over 1200 DNA fingerprints of V. vinifera, rootstocks and hybrid cultivars. Seventeen vinifera cultivars (Abouriou, Brachet 2, Cep rouge, Charmont, Courbu, Kadarka, Kóztegalo, Lercat, Mayolet, Médoc noir, Muscat fleur d'oranger, Plavai, Saint Fiacre, Sainte Marie, Salicette, Sicilien faux, and Verdot) shared a least one allele per locus with Norton at 6 of the 7 loci (Table 1a) in this broad assessment, but the frequencies of the shared alleles were high within the species (being found in 11% to 34% of all tested vinifera cultivars).

Failure to find actual parents led us to evaluate 'Norton' allele frequencies within 'Vitis' subgroups to provide inferences on 'Norton' parentage. There were 181 Euvitis of North American origin and 354 V. vinifera cultivars for which there were data on SSR loci selected for this study, and further analyses focused on these data. Frequency of alleles observed in 'Norton' were assessed within these groups, as well as calculating allelic frequencies within individual species (data not shown) and series among North American Euvitis (Table 2). Although the sample size is small for the Euvitis species and hybrids from North America, this material represents a broad cross-section of diversity and these data likely include a large percentage of all common alleles. 'Norton' contained alleles at 6 of the 13 loci which were never observed in the extensive V. vinifera data but were present in the North American Euvitis data set, suggesting that these were likely contributed by a non-vinifera parent. By extension, if 'Norton' had a V. vinifera parent, the remaining 'Norton' alleles at these loci were likely contributed by vinifera. These non-vinifera alleles were all present in the series Aestivales, which supports the possibility that 'Norton' is a V. aestivalis x V. vinifera hybrid, though few alleles were completely absent from any North American Euvitis species data sampled, and only the frequency of allele 237 in locus VVMD7 was markedly greater in the Aestivales series than all other North American Euvitis series studied.

Both 'Norton' alleles at VVMD36 were observed at relatively low frequencies in this data set, suggesting that genotypes with one of these alleles and with matches to other 'Norton' alleles at other loci, might represent close ancestors (or descendents) of 'Norton' or its parents. Two vinifera cultivars in the database contained one of the two 'Norton' alleles at VVMD36 and were further observed to match 'Norton' alleles at 9 of the 12 additional loci (Table 1b). These cultivars, 'Milgratan' and 'Enfariné noir', are obscure French grapes which might have been included among the "mixed French grapes" reported to be grown by Dr. Norton in 1822 (Ambers and Ambers, 2004). Even more intriguingly, 'Enfariné noir' means "floured black" reflecting the powdery bloom on the leaves and black berries of this cultivar, remarkably similar in appearance to those of 'Pinot Meunier' (this flour covered appearance explains the name Meunier, which is French for "flour"). The 'Stark-Star' described by Hedrick (1908) as a likely 'Catawba' by 'Hermann', appears clearly not a parent of 'Norton' (Table 1a) as proposed (Prince, 1830). Even more intriguingly, 'Enfariné noir' means "floured black" reflecting the powdery bloom on the leaves and black berries of this cultivar, remarkably similar in appearance to those of 'Pinot Meunier' (this flour covered appearance explains the name Meunier, which is French for "flour").

Table 1a. Alleles for 'Norton' and other significant cultivars discussed in this paper including: the 17 vinifera cultivars (out of a database of 744) which share 6 of 7 alleles with 'Norton' (Note 1), an alleged parent of 'Norton' that does not have alleles supporting this parent progeny relationship (Note 2), and a reported possible hybrid of 'Norton' that does not have alleles supporting this parent progeny relationship (Note 3).

<table>
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<th>VVMD31</th>
<th>VVS2</th>
<th>VrZAG62</th>
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Table 1b. Profiles of 13 SSRs for ‘Norton’, various vitis vinifera cultivars (6 of the 354 evaluated) and the hybrid ‘Gold Coin’ (DVITO061), a T.V. Munson hybrid, reported to have ‘Norton’ as a parent (Hedrick, 1908). Two vitis vinifera cultivars, Enfariné noir and Milgranet, share rare alleles with ‘Norton’ at VVMD36.

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Table 2. Alleles for ‘Norton’ grape at 13 SSR markers and frequency of those alleles within 354 Vitis vinifera cultivars and 181 Euvitis species/hybrids from North America in the database, for which there were data at all 13 SSR markers. Allele frequencies for North American species are further divided by series.

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No known grape collection includes an accession called the 'Bland' grape, though it was listed in the Prince Nursery catalogues from 1822 through 1861 and was described by Downing (1847) as “one of the best of our native grapes, approaching in flavor and appearance, the Chasselas grapes of Europe” which were the leading table grapes of the time. There is considerable confusion over ‘Bland’. Indeed, Hedrick (1908) indicates some authorities considered ‘Bland’ a likely hybrid of vinifera because of its resemblance to ‘Chasselas’ and that “Bolling... tells of the origin of the Bland grape, which we now know to be a native, and wrongly (saying) ... it grew from the seed of a European raisin”. Most recorded comments on ‘Bland’ suggest it is a variety or cultivar, however, there was a species designation, *Vitis blanda* which was referred to as “the Bland grape” by Rafinesque (1830) and who indicated that there were many varieties, while Hedrick (1908) lists *Vitis blanda* as a synonym for *Vitis labrusca*. Requests from public and private grape breeders have not unearthed this reported seed parent of ‘Norton’, though further SSR analyses may yet reveal the ‘Bland’ grape, hiding under another name. If so, its identification may be strengthened through having SSR alleles which are consistent with being a parent of ‘Norton’.

Dendrograms resulting from genetic distance analyses were consistent with *V. vinifera* and North American species contributing to the ancestry of ‘Norton’. When analysis was conducted on 175 Euvitis of North American origin and a subset of 40 diverse *V. vinifera* cultivars for which there were complete data at all 13 SSR loci (data not shown), ‘Norton’ clustered into a large group comprised of 17 *V. aestivalis* accessions, 4 *V. cinerea* accessions, and 1 *V. labrusca* accession. This cluster was distinct from the tightly clustered *V. vinifera* accessions. Reanalysis excluding the vinifera had little affect (data not shown). Cluster analysis can be markedly influenced by having a large number of related accessions, so a further analysis was conducted on a balanced group of 49 accessions including 6 diverse accessions from each Euvitis series in North America and Europe plus hybrid cultivars known to include ancestry of North American species. In this analysis ‘Norton’ clustered into a group otherwise comprised completely of *V. vinifera* cultivars and known or speculated *V. vinifera* or ‘Norton’ hybrids, while all North American species accessions grouped into other distinct clusters, with the *V. aestivalis* accessions in the most closely allied cluster (Fig. 2).

A study conducted concurrently with this project is reported only in a thesis (Parker, 2007) and is not accessed by conventional literature searches, but is deserving of mention. The focus of the
project was use of SSR analysis and morphological data to assess the pedigree of "Cynthiana"/"Norton". In that study, 12 SSRs were used to evaluate 'Norton', 'Cynthiana', a locally collected sample of "southern V. aestivalis", and multiple accessions of each of the following bulked into one sample for each group: V. labrusca, V. riparia, V. aestivalis, and the vinifera cultivar 'Chasselas'. 'Norton' and 'Cynthiana' were reported to be genetically identical by SSR analysis. Due to shared loci at several alleles, the author concluded that "Vitis aestivalis (northern accessions), Vitis labrusca, and 'Chasselas' (Vitis vinifera) varieties are involved in the parentage of 'Cynthiana'/'Norton'". The data presented in this thesis are useful, but represent exploration of very few Vitis genotypes, and this conclusion in essence only excludes V. riparia as a 'Norton' ancestor among the study accessions, since the other material shared loci with 'Norton' at only 2 to 5 alleles of the 12 tested. 'Cynthiana' and "southern V. aestivalis" were also reported to resemble each other in leaf and shoot morphology.

In conclusion, the data evaluated in our study could not be used to identify likely parents of 'Norton', though a combination of SSR data and circumstantial evidence provide tantalizing support that 'Enfariné noir' may be a close relative. All resulting data are consistent with V. vinifera and V. aestivalis contributing substantially to the genetic background of 'Norton'. It is further proposed that initial focus on the rare alleles of 'Norton' at locus VVMD36 may provide a valuable tool in ultimately identifying the parents of 'Norton'.

**Literature Cited**


