



Identification of Unknown *Vitis* accession using Morphology and Genetics

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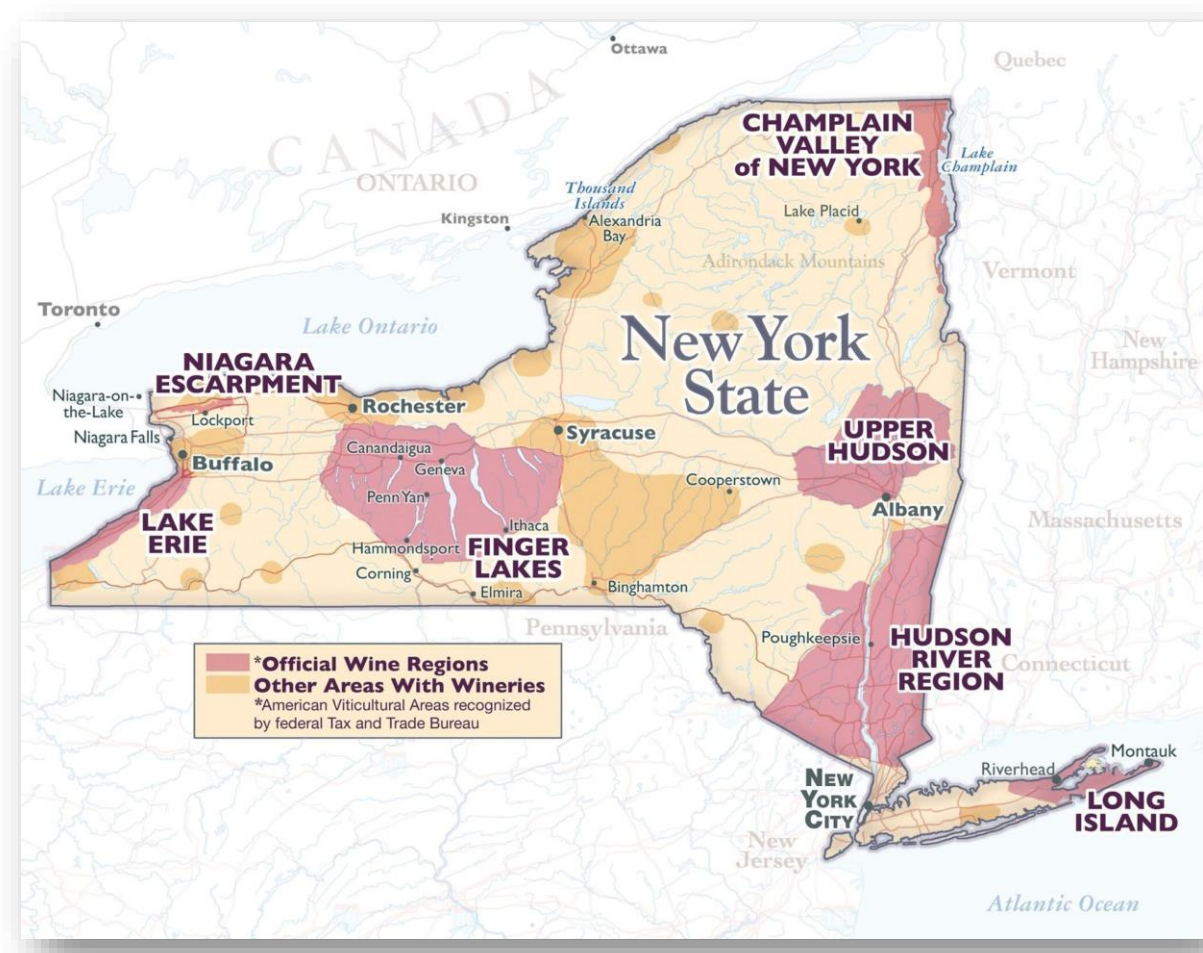


Introduction

- The unique and nuanced morphology of grapevines across species makes a dichotomous key for the untrained eye nearly impossible. The ability to identify grape cultivars by sight is a great feat of experience and expertise
- When an unknown *Vitis* accession was gifted to the PGRU (Plant Genetics Resources Unit), a project was outlined to use morphological and genetic methods, as well as historical knowledge to attempt to identify the variety in question
- New accessions are added to the PGRU collection to capture breeding and cultivar history, along with safeguarding genetic resources for crop security.

History

- There is an extensive history of grapevine cultivation in New York State, since the settlement of Europeans in the sixteenth century.
- Unrestrained hybridization and open pollination has created many unique accessions to slip through the records and fade into obscurity
- The mystery grapevine was found in Hudson Falls, New York, a town in the upper Hudson Valley Region of the state



Discussion and Conclusion

- Exploring the morphology of the mystery vine compared to our selected vines provided lots of insight into the categorization of the four major grape species
- The morphological points of study indicated that this mystery grape variety contains many traits strongly associated with *Vitis labrusca* and *Vitis vinifera*
- The gene sequencing results will further develop our understanding of the mystery cultivar and allow for a pedigree to be constructed
- This project has significance because unknown grape varieties contain within them the potential to fill gaps in the current knowledge of cold hardy grapevine cultivation in a variety of ways (disease resistance, fruit quality, winemaking, characterization, etc.)
- Making a project like this is a step towards a more comprehensive, agricultural knowledge that has the potential to benefit the world.

Method of Study: Genetics

- A variety of *Vitis* spp. and hybrid vines were collected in the USDA PGRU grapevine repository in Geneva, NY. This included three pure *Vitis* species (*V. labrusca*, *V. riparia*, *V. aestivalis*) that grow natively in the region and the cultivated grape species (*V. vinifera*). The hybrids selected originated in the region of our mystery grapevine or have similar background to lost cultivars from the area³. (Table 1)

- DNA was extracted from each of these accessions. (Figure 1)
- This DNA is being used in PCR with 16 selected SSR markers^{1,2,5}, which can be cross referenced with each other on the *Vitis* International Variety Catalogue (VIVC) and the GRIN-Global databases. (Data being produced.)
- The mystery sample's DNA is also going to be sequenced using (RhAmpseq), which will provide a much greater depth into the genetic code of the mystery cultivar, allowing for a parentage to be revealed

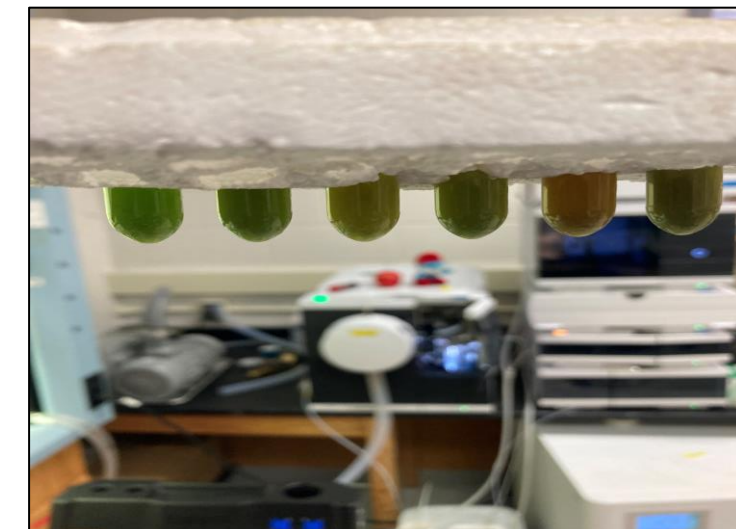


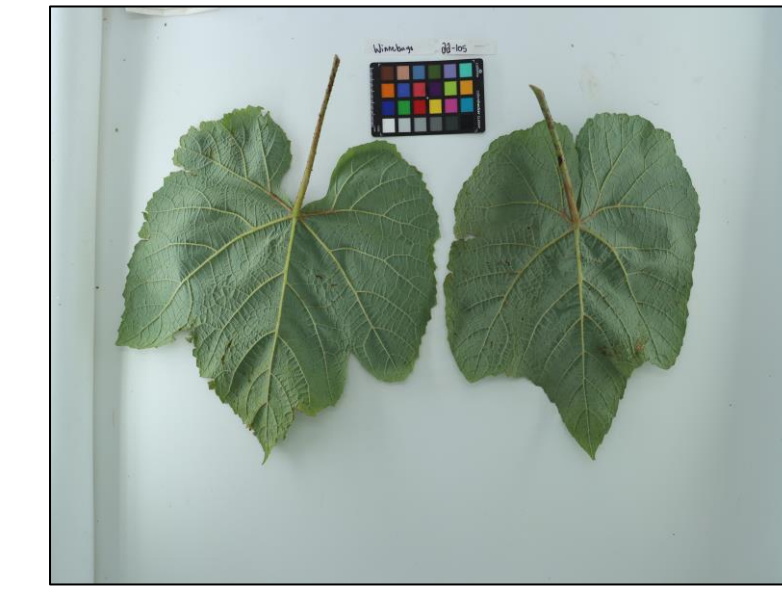
Figure 1 shows microcentrifuge tubes during the early steps of DNA Extraction

Table 1 Selected accessions from the PGRU grape repository to compare to the "mystery grape".

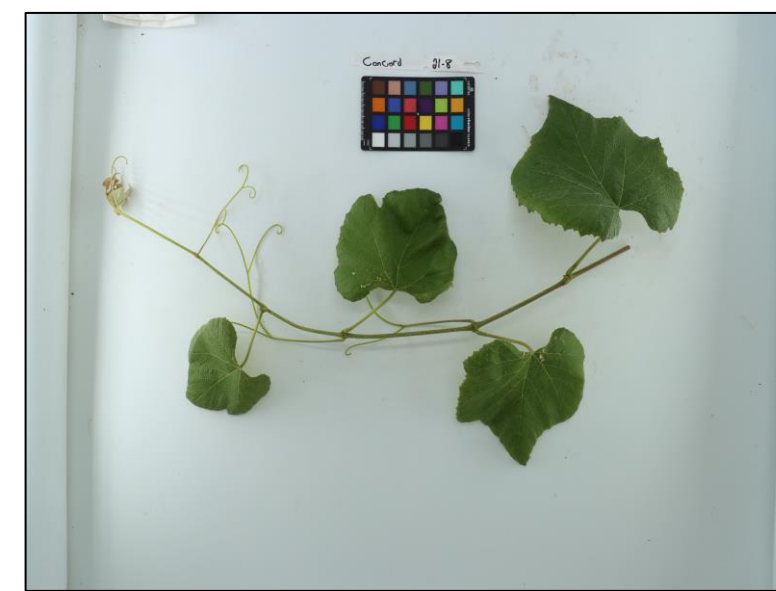
#	Name	Cultivar Type	If Hybrid, Species in Background
1	Isabella	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i>
2	Eumelan	hybrid	Isabella (open pollinated) O.P.
3	Oconee	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i> , unknown O.P.
4	Clinton	hybrid	<i>V. riparia</i> , <i>V. labrusca</i>
5	Black Eagle	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i>
6	Downing	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i> , maybe <i>V. aestivalis</i>
7	Iona	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i>
8	Niagara	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i> , unknown O.P.
9	Lutie	<i>V. labrusca</i>	
10	Champagne	<i>V. labrusca</i>	
11	Winnebago	<i>V. aestivalis</i>	
12	Bougher	<i>V. riparia</i>	
13	REM 48-77	<i>V. aestivalis</i>	
14	John Viola	<i>V. riparia</i>	
15	Feteasca Neagra	<i>V. vinifera</i>	
16	Concord	hybrid	<i>V. labrusca</i> , <i>V. vinifera</i>
17	Mystery Grape	Unknown	

Method of Study: Ampelography

- Shoots with meristems, immature and mature leaves, and immature fruit clusters of the 16 selected varieties from the repository were compared to the mystery grapevine.
- Fourteen morphological traits were selected to compare across the accessions: leaf shape, petiole sinus shape, meristem pubescence, meristem anthocyanins, leaf pubescence (immature and mature; adaxial and abaxial), leaf margin, lobe count, lobe sinus shape, tendril arrangement, and grape cluster shape and density.
- Using ampelographic (i.e., morphology) traits⁵ of these vines using demonstrated the nuance of grapevine phenotypes and allowed for the collection of specified data points that distinguish the selected cultivars from each other.



Figures 2-5 The abaxial side of leaves. Underside color is affected by the density and color of leaf hairs. This also highlights the variety of leaf shapes, lobe quantity, petiole and lobe sinuses shapes and depths, along with leaf margin patterns across the accessions.



Figures 6-9 Shoots and meristems of four selected grapevine accessions. This highlighted meristem pubescence, anthocyanin color, and tendril arrangements.



Figures 10-13 Immature grape clusters of four of the same selected vines, demonstrating the differences in cluster structure including shape (conical v. ovular), density (loose vs. tight), the presence or absence of a shoulder.

Table 2 Ampelographic traits compared to the "mystery grape" (#17). Highlighting shows accessions with the same trait as the mystery accession.

#	Meristem Pubescence	Presence of Anthocyanins on Meristem	Adaxial Leaf Pubescence (immature)	Adaxial Leaf Pubescence (mature)	Abaxial leaf Pubescence (immature)	Abaxial leaf Pubescence (mature)	Leaf Shape	Petiole Sinus	Lobe Sinus Depth	Lobe Count	Leaf Margin	Tendril Arrangement	Grape Cluster Density	Grape Cluster Shoulder
1	Very Dense	Yes (Orange)	Dense	Sparse	Dense	Dense	Pentagonal	Open V	Shallow	5	Straight Teeth	Discontinuous	Loose	No
2	Very Dense	Yes (Pink)	Medium	Medium	Dense	Medium	Wedge Shaped	Open V	Very Shallow	5	Straight Teeth	Discontinuous	Loose	No
3	Dense	Yes (Orange)	Sparse	Sparse	Dense	Medium	Pentagonal	Open V	Very Deep	5	Straight Teeth	Discontinuous	Loose	No
4	Dense	Yes (Pink)	Dense	Sparse	Dense	Sparse	Wedge Shaped	Open U	Shallow	3	Concave	Discontinuous	Dense	No
5	Very Dense	Yes (Orange)	Sparse	Sparse	Very Dense	Very Dense	Pentagonal	Open U	Very Deep	5	Concave	Discontinuous	Loose	No
6	Dense	No	Sparse	Medium	Dense	Dense	Wedge Shaped	Open U	Very Shallow	3	Convex	Continuous	Dense	Yes
7	Very Dense	Yes (Orange)	Medium	Medium	Very Dense	Very Dense	Pentagonal	Wide Open U	Medium	5	Convex	Discontinuous	Loose	No
8	Very Dense	Yes (Pink)	Medium	Sparse	Very Dense	Very Dense	Pentagonal	Open V	Medium	5	Concave	Discontinuous	Dense	Yes
9	Very Dense	Yes (Orange/Pink)	Very Dense	Very Dense	Very Dense	Very Dense	Wedge Shaped	Wide Open V	Very Shallow	3	Concave	Continuous	Dense	Yes
10	Very Dense	Yes (Orange)	Medium	Medium	Very Dense	Very Dense	Pentagonal	Open V	Shallow	5	Concave	Discontinuous	Dense	No
11	Very Dense	Yes (Very Pink)	Sparse	Sparse	Very Dense	Dense	Wedge Shaped	Open U	Very Shallow	3	Convex	Discontinuous	Loose	No
12	None	None	None	None	Medium	Sparse	Wedge Shaped	Wide Open U	Very Shallow	3	Hook Style	Discontinuous	Dense	Yes
13	Very Dense	Yes (Orange/Pink)	Dense	Sparse	Very Dense	None	Pentagonal	Open U	Medium	5	Straight Teeth	Continuous	Male (no fruit)	Male (no fruit)
14	None	None	None	None	None	None	Wedge Shaped	Wide Open U	Medium	3	Hook Style	Discontinuous	Dense	Yes
15	None	Yes (Crimson)	None	None	None	None	Pentagonal	Open V	Deep, closed	5	Hook Style	Discontinuous	Dense	No
16	Very Dense	Yes (Orange)	Very Dense	Medium	Very Dense	Dense	Wedge Shaped	Wide Open U	Very Shallow	3	Concave	Discontinuous	Loose	Yes
17	Very Dense	Yes (Orange)	Medium	Sparse	Very Dense	Dense	Pentagonal	Wide Open U	Shallow	5	Concave	Discontinuous	Loose	No

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