

Nuclear and chloroplast sequences resolve the Concord grape mystery

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Introduction



Bull's original Concord grape vine in Concord, MA. Photo courtesy of J. Wen.

The Concord grape is both culturally and commercially important in the United States as a source of juice, jelly, jam, and wine as well as a popular cultivated plant. However, the genetic heritage of the Concord grape has been shrouded in mystery since its development by Ephraim Bull in 1849. An avid horticulturalist, Bull experimented with some 22,000 grape plants in order to develop the perfect, cold-hardy crop with bisexual flowers. While some sources report that the Concord grape was derived from selection from wild *Vitis labrusca* in Concord, Massachusetts, others argue that it is a hybrid of two or more grape species (Wepman 2010; Moore & Wen 2014).

The purpose of this study is to test the following hypothesis:

The Concord grape is the result of a hybridization among two or more grape species, most likely with *Vitis labrusca* as the maternal parent and with *V. aestivalis* and *V. vinifera* as the primary paternal parent suspects.

Contained within the Vitaceae family, the grape genus, *Vitis* L., includes about 65 species worldwide (Wen 2007), 9 of which are found in the mid-Atlantic region of the United States: *V. aestivalis*, *V. baileyana*, *V. labrusca*, *V. cinerea*, *V. vulpina*, *V. rotundifolia*, *V. riparia*, *V. vinifera*, and *V. rupestris* (Moore & Wen 2014).

Methods

Molecular Experiments:

- In order to achieve the most complete analysis, grape specimens were collected from across the entire mid-Atlantic region (where Bull would have had access). Outgroup species from other regions were also included.
- DNA was extracted from 59 different samples of the *Vitis* genus using Autogen and the CTAB buffer at the Museum Support Center.
- Samples were processed through PCR, gel electrophoresis, ExoSap purification, and Sanger sequencing in the Laboratories of Analytical Biology at the National Museum of Natural History.

Ten different molecular markers were used, including six plastid (*matK*, *psbA-trnH*, *petN-trnC*, *ycf1*, *trnLF*, and *trnS-G*) and four nuclear (*AT103*, *sqd1*, *phyA*, and *GAI1*) to explore both the maternal and paternal history of the Concord grape.

Details on the primers used in PCR and on the sequencing and DNA purification procedures can be found in Wen et al. (2007), Li et al. (2008), and Lu et al. (2013).



Southwest Virginia Grape Collecting Trip: (A) *V. vulpina*, (B) *V. baileyana*, (C) New *Vitis* hybrid, (D) Collecting wild grapes in the field, (E) *V. aestivalis*.

Photos courtesy of J. Wen and S. Herron.

DNA Sequence Assembly and Phylogenetic Analyses:

- Using Geneious 7.1.5 (Drummond et al. 2014), all sequences were checked for inaccuracies and aligned.
- jModeltest 2.1.4 (Darriba et al. 2012) was used to find the best -fit model for phylogenetic analysis.
- MrBayes 3.2.2 (Ronquist et al. 2012) and SplitsTree 4.13 (Huson & Bryant 2006) were used to generate the phylogenetic trees.
- The plastid and nuclear data were analyzed as separate matrices.

Results – 1. Nuclear Data

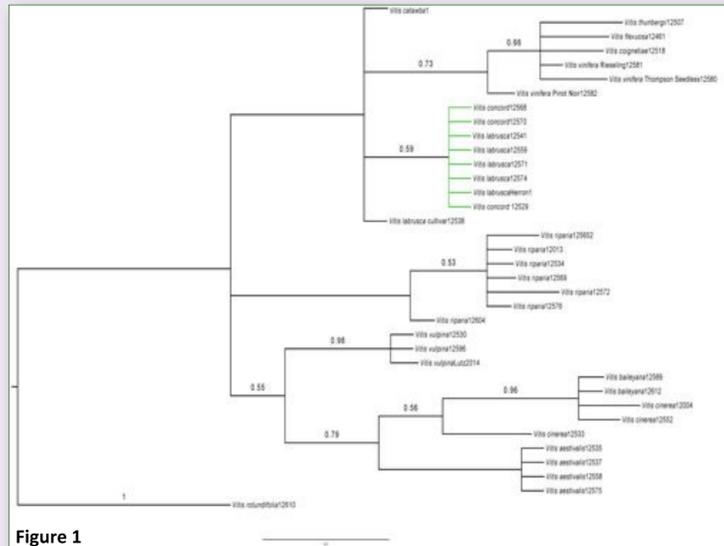


Figure 1

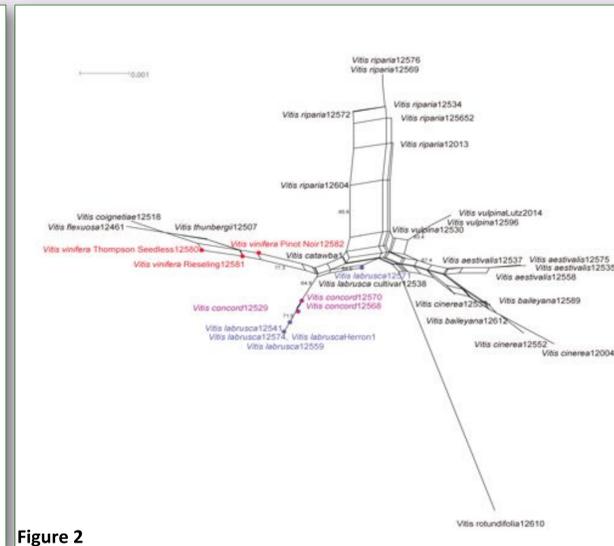


Figure 2

Results – 2. Chloroplast Data

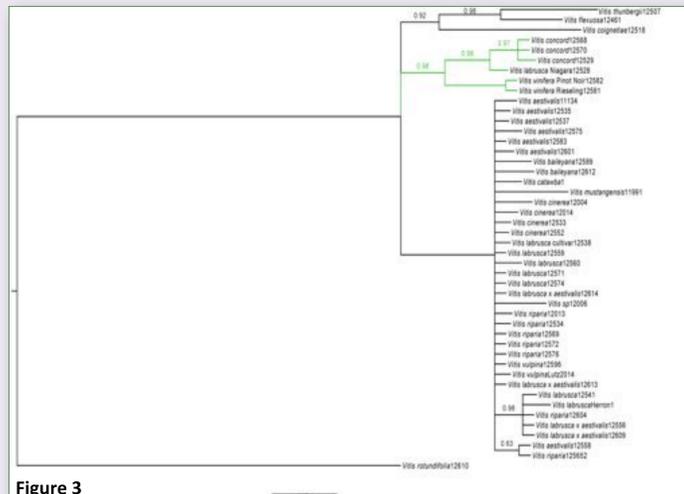


Figure 3

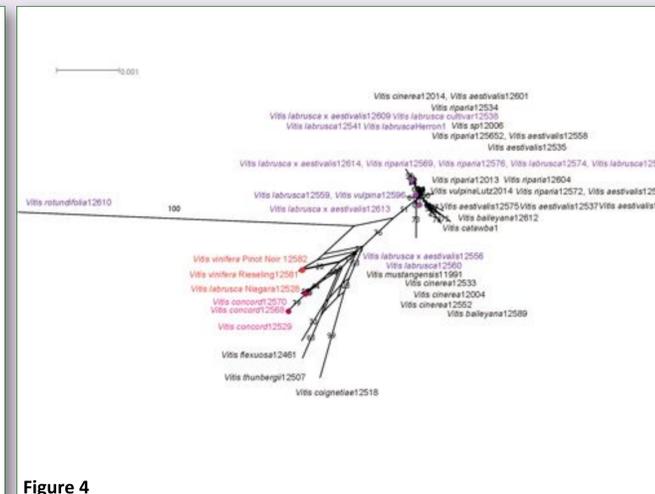


Figure 4

Figures 1 and 3: Phylogenetic trees based on Bayesian inference with MrBayes 3.2.2 using nuclear (Figure 1) and plastid (Figure 3) data. Above each branch are the posterior probabilities for the node. The Concord-containing clade is highlighted in green. Unlabeled branches have a posterior probability of one.

Figures 2 and 4: SplitsTree 4.13 analyses of the data matrices for the nuclear (Figure 2) and plastid (Figure 4) markers, using neighbor-net method. Each branch represents a clade of organisms, and thickness of the branch represents species variation. SplitsTree analyses are courtesy of Yunjuan Zuo.

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Results – 3. Sequence Pattern

Nuclear				Plastid			
<i>AT103</i>	<i>GAI1</i>	<i>phyA</i>	<i>ycf1</i>	<i>matK</i>	<i>petN-trnC</i>	<i>psbA-trnH</i>	<i>trnL-F</i>
444 bp	961 bp	514 bp	324 bp	808 bp	790 bp	445 bp	855 bp
bp 95	bp 716		bp 178 & 179	bp 203	bp 387-440	bp 234	bp 558-562
bp 110	bp 889				bp 561		bp 587
bp 267					bp 588		bp 722
bp 342							bp 827
							bp 833
							bp 836

Table 1: This table shows some of the informative loci within each gene region. Blue signifies unique sites shared by Concord and *V. labrusca*; red indicates unique sites shared among Concord, *V. vinifera*, and *V. labrusca*; and green signifies unique matches between Concord and *V. vinifera*.

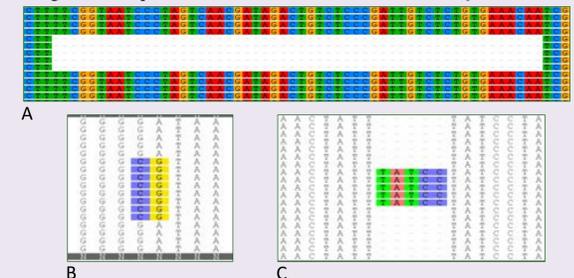
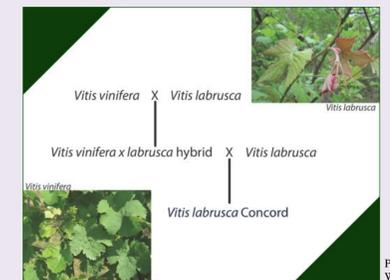


Figure 5: (A) *petN-trnC* spacer: A shared deletion of 53 base pairs between the Concord grape and *V. vinifera*. (B) *sqd1* gene: Two sites shared by Concord and *V. labrusca*. (C) *trnL-F* region: An insertion of 5 base pairs shared by Concord and *V. vinifera*.

Conclusions

- The chloroplast data show that the European *V. vinifera* and the Concord grape form a clade and share nearly identical sequences, indicating that *V. vinifera* was in fact the original maternal species from which Concord was derived. A relationship between the Concord grape and *V. vinifera* was long suspected due to their shared bisexual characteristic, a quality originally exclusively found in *V. vinifera*.
- The strong similarity between Concord and *V. labrusca* in nuclear sequences establishes *V. labrusca* as the paternal parent of the Concord grape. The distinct correlation between *V. labrusca* and Concord grape sequences, as well as morphological similarities, indicates that the Concord hybrid was backcrossed with *V. labrusca* in its development by Bull.
- Sawler et al. (2013) reported that the Concord grape contains c. 30% of the *V. vinifera* genome. Based on this combined evidence, the backcrossing occurred once; in this scenario, the Concord grape would contain 75% of *V. labrusca* genome and 25% *V. vinifera* genome.



Photos courtesy of J. Wen and S. Herron.

Figure 6: Ephraim Bull's breeding scheme for the Concord grape, based on our chloroplast and nuclear sequence comparisons.

Future Research Directions

- In addition to the Concord grape, several other important grape cultivars have controversial ancestry, which still requires extensive exploration.
- This study revealed a close taxonomic relationship between *V. cinerea* and *V. baileyana*; their taxonomic status needs further investigation.
- In general, the role of hybridization in *Vitis* speciation awaits future research.

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