A phylogeographic approach to resolving the relationships and distribution of North American *Parthenocissus*

Smithsonian Institution
National Museum of Natural History

Matt Chansler, Michigan State University; Assisted by Joshua Springer, Michigan State University;

Advised by Dr. Elizabeth Zimmer and Dr. Jun Wen, NHRE National Museum of Natural History

Introduction

~***P****arthenocissus* is a common genus of climbing or creeping plants in the grape family, Vitaceae. Its distribution includes the Eastern half of North America as well as Southern and Eastern Asia. The three species in North America, *P. quinquefolia*, *P. vitacea*, and *P. heptaphylla* show morphological variation (Figure 1) as well as geographic predilection.

~***N****orth American Parthenocissus* vary morphologically both within and between species. Relatively high morphological intraspecific variation gives us cause to use genetic information to clarify population level variation. Chloroplast DNA includes spacer regions that do not code for proteins. As a result, they mutate more rapidly and are useful for population-level analyses. The utility of plastid markers is well-known. *trnH psbA* is a short, easily sequenced barcoding marker common to most dicots. *trnC petN* is longer and has been used to classify *Panax*.

~***G****enetic data is also useful at another level. With the end of the Pleistocene Ice Age, *P. quinquefolia* and *P. vitacea* expanded northward, acquiring the range they occupy today. By combining phylogenetic data with geographic information, we attempt to explain how the genus redistributed from refugia after the glacial retreat.

Figure 1. From left to right, *P. quinquefolia, P. heptaphylla, P. vitacea*.

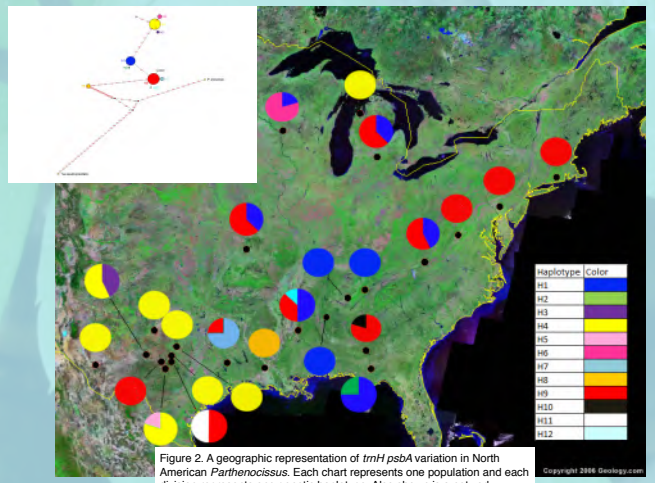

Figure 2. A geographic representation of *trnH psbA* variation in North American *Parthenocissus*. Each chart represents one population and each division represents one genetic haplotype. Also shown is a network describing the relative abundance and relatedness of each haplotype, based on the same *trnH psbA* sequences.

Methods

We collected specimens from populations of all three species (Figure 3). DNA was **extracted** with both the Quiagen DNEasy® kit and the Autogen® automated nucleic acid extractor. DNA generated via Quiagen was **diluted** to 0.1X, while DNA generated via Autogen was diluted to 0.01X. Two regions of the chloroplast genome, the *trn*H-*psb*A barcoding marker and the *trn*C-*pet*N intergenic spacer were **amplified** using polymerase chain reaction. The product was then **ExoSapped** to destroy leftover nucleotides and primers. ExoSap product was used for **cycle sequencing**. After purification through Sephadex, cycle-sequencing product was submitted to the National Museum of Natural History Laboratory of Analytic biology and **sequenced** with Applied Biosystems' ® 3730xl capillary sequencer.

Editing and alignment were performed in Geneious® and Se-Al, while PAUP was used to construct a phylogenetic tree. Figtree and Mesquite were used to edit the tree. Dnasp and Network were used to create a network diagram .

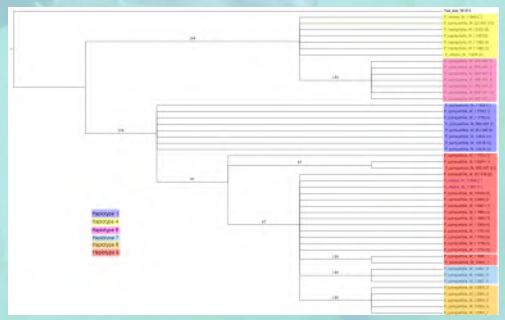

Figure 3. A majority-rules semi-strict consensus tree created with concatenated *trnH psbA* and *trnC petN* sequences. The outgroup used was *Yua austroorientalis*.

Results

- Less variation appeared within populations than expected.
- *trnH psbA* was more variable than *trnC petN*.
- Surprisingly, there was little to no genetic distinction between *P. vitacea* and *P. heptaphylla*, even considering both *trnH psbA* and *trnC petN* .
- There was, however, variation between populations within the same species.
  - **Haplotypes 1** and **9** comprised the majority of *P. quinquefolia* and were both present at many populations
  - **Haplotype 8** was found at only one site in Louisiana. The distinction between *P. quinquefolia* from this site and the rest of the species was well-supported by both markers (Figure 3).
  - Both markers also supported the separation of **haplotype 3**, found only in north-western Texas, **haplotype 6**, found only in Wisconsin, and haplotype 7, found only in eastern Texas.
- Interspecific variation did have a geographic component.
  - **Haplotype 9** was more common in the northeast and in Texas, while **haplotype 1** appeared more frequently in the central part of the country.
  - Infrequent variations such as **haplotypes 5, 10**, **11**, and **12** were shown to be genetically very close to the major haplotypes with which they co-occurred (Figure 2).

Conclusions

- With even one small barcoding marker, it is possible to draw a number of conclusions about past and present distribution of *Parthenocissus.*
- Morphologically, this genus shows a great deal of variation. This variation does not always, however, carry over to variety of haplotypes.
  - *P. heptaphylla* and *P. vitacea*, fairly distinctive in terms of appearance and habit, were almost completely identical genetically where they co-occurred.
  - *P. quinquefolia* from Louisiana, represented by **haplotype 8**, are an example of morphological variation that *did* correspond to genetic separation. Plants from this site had especially prominent leaf dentation and pubescence.
- **Haplotype 1** did not appear in Texas at all, while **haplotype 9** did. **Haplotype 9** also appears throughout the northeast and the Midwest. Because Texas is a center for Vitaceae, as well as *Parthenocisssus* diversity, **haplotype 9** is probably the original migrant that radiated from Texas after glaciation**. Haplotype 1**, therefore, is likely a more recent divergence.
- **Further research will include sequencing nuclear markers and more plastid markers as well as microsatellites if necessary.**

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