Evolution of the deciduous southeastern azaleas (*Rhododendron* subgenus *Pentanthera* section *Pentanthera*)

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Introduction

- Genus *Rhododendron*

  ~1,000 species of woody plants

  Habitat generalists

  Section *Pentanthera*: 17 species in Asia, North America, and the Caucasus

  Subsection *Pentanthera*: southeastern azaleas

- Importance of southern azaleas

  Horticultural importance:

  Numerous societies dedicated to breeding distinct species

  Economic importance:

  Consumers interested in buying/selling seeds of specific varieties

  Historical biogeography

- Good group for comparison to morphological phylogeny (Figure 1)

- Project Goals:

  Use molecular data to build a phylogeny of deciduous azaleas

  Determine if subsection *Pentanthera* is monophyletic

  Determine species-level relationships within *Pentanthera*

  Analyze placement of new species *Rhododendron colemanii*

  Compare topology of molecular phylogeny to morphological

  Generate molecular data that can be used for future projects

Methods

. DNA extraction via Qiagen DNeasy Plant Mini kit

. PCR amplification of chloroplast (Figure 2, following Shaw et al., 2007) and
nuclear markers (following Evans et al., 2000)

. Amplicons gel-extracted via Qiagen Gel Extraction kit

. Sanger sequencing at Nevada Genomics Center (Reno, NV)

. Automated alignment via Geneious v.8

. Analysis of individual DNA regions and combined molecular matrices within
Parsimony (PAUP) and Maximum Likelihood (RAxML) framework
(Stamatakis et al., 2008)

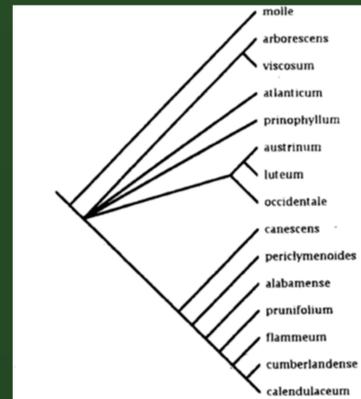*Rhododendron prinophyllum*

Photo by: Emily Gillespie

Fig. 1. Strict consensus tree of 16 most parsimonious trees generated from morphological data of *Rhododendron* sect. *Pentanthera* (Kron 1993).
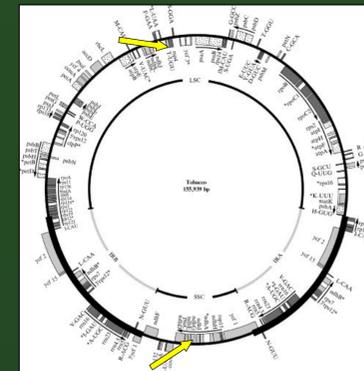

Fig. 2. *Nicotiana tabacum* (tobacco) chloroplast genome. Arrows indicate positions of trnV-ndhC and rpl32-trnL intergenic spacers
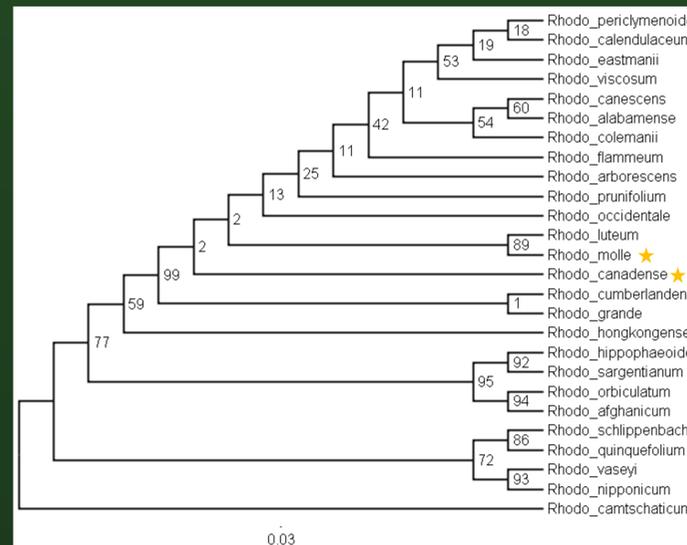
Results

Figure 3. Maximum Likelihood phylogeny generated using single intergenic region psbD-trnT.
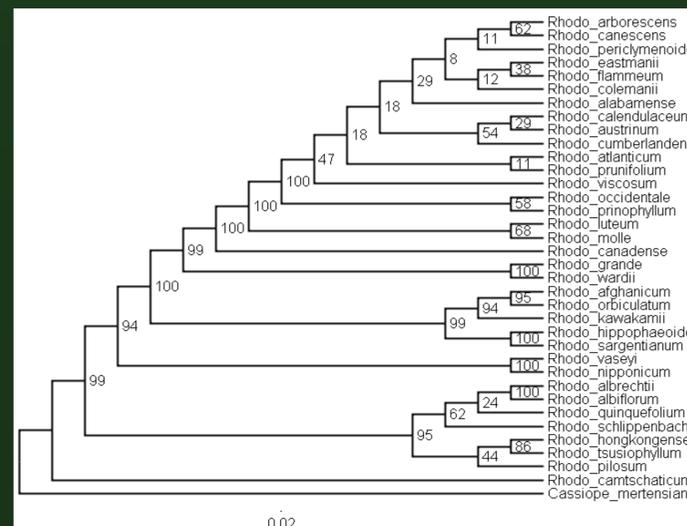

Figure 4. Maximum Likelihood phylogeny generated using four nuclear genes and six chloroplast genes.

Conclusions

. The amount of statistical support for the phylogeny developed using all
the available genes is much higher than using a single gene (Figures 3,
4).

. Based on the data we've gathered so far, the subsection *Pentanthera* appears to be paraphyletic, since *R. molle* is also included (Figure 4).

. Clades such as the one containing *R. hippophaeoides, R. sargentianum,
R. orbiculatum,* and *R. afghanicum* in the tree generated using a single
dataset (Figure 3) are further supported by the addition of data from other genes and intergenic spacers (Figure 4). This shows the need for using
multiple genes/gene regions when constructing and studying phylogenetic relationships.

. There is strong disagreement between the phylogeny developed using
morphology (Figure 1) and the molecular data (Figure 4). This indicates
the importance of collecting additional molecular data in the future and
its use in building phylogenies. It also suggests that using flower color as
a character for morphological trees is not as reliable as what was previously believed.

Future Plans

. Sequence additional chloroplast and nuclear genes to increase resolution

. Analyze phylogenies generated using chloroplast and nuclear genes
separately

. Analyze sequences using Mr Bayes maximum likelihood inference

. Compare phylogenies generated using PAUP, RAxML and Mr Bayes

Literature Cited

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Acknowledgments