The Genetic and Taxonomic Aspects of Penstemon

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INTRODUCTION

The classification of organisms (taxonomy) is one of the most ancient practices in biology, having its roots in ancient Greek. Through comparative morphology, species can be identified as belonging to natural groups, arranged hierarchically. This hierarchy is commonly used to determine the evolutionary relationships within any taxonomic group, known as a phylogeny. One method, for constructing phylogenies uses visible physical characters, subsumed under the term morphology, looking for similarities and differences of form and structure which unite individuals. The other method applies the modern techniques of genetic analysis to compare genetic information, and is known as molecular phylogenetics. The principle of this method is to find the tree which best explains the observed sequence data given an explicit stochastic model of molecular evolution. For this project, a molecular approach was taken to create a phylogeny within the *Penstemon* genus. *Penstemon* (tribe Cheloneae: Scrophulariaceae) is a large genus (about 275 species) of perennial plants endemic to North America, ranging from Alaska to Guatemala (large genus of perennial plants endemic to North America). To date, the *Penstemon* phylogeny has divided the genus into six subgenera : *Penstemon*, *Habroanthus Saccanthera*, *Dasanthera*, *Cryptostemon (monotypic)* and *Dissecti (monotypic)*, based on their anther characters. The objectives of our study are: (1) to construct a molecular phylogeny to compare back to the existing morphological one; (2) look at evolutionary origin of important floral characteristics within the genus. We have generated nucleotide and sequences of ITS region to determine the evolutionary relationship between these Penstemon.

P. debilis

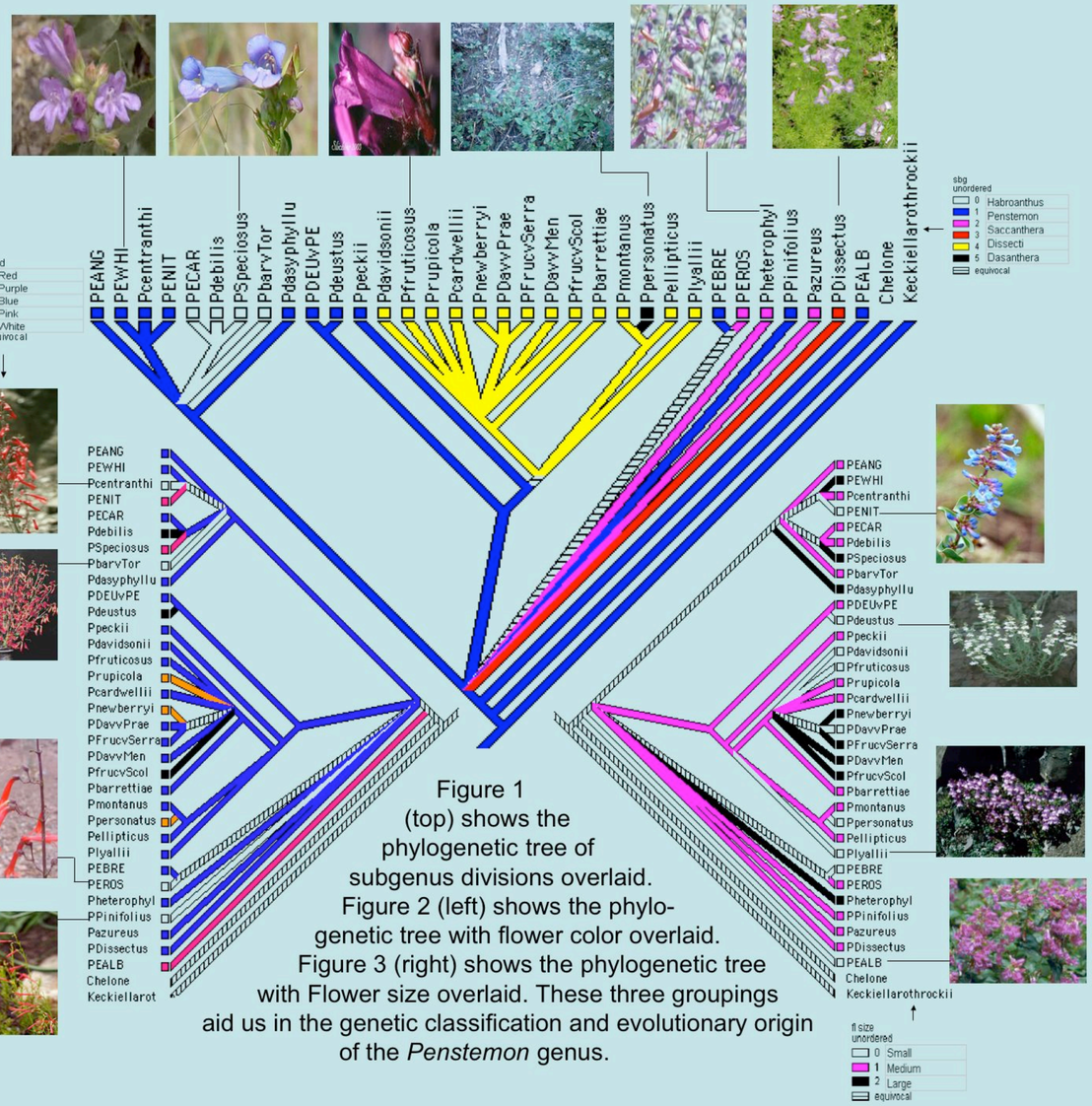


Figure 1 (top) shows the phylogenetic tree of subgenus divisions overlaid. Figure 2 (left) shows the phylogenetic tree with flower color overlaid. Figure 3 (right) shows the phylogenetic tree with Flower size overlaid. These three groupings aid us in the genetic classification and evolutionary origin of the *Penstemon* genus.

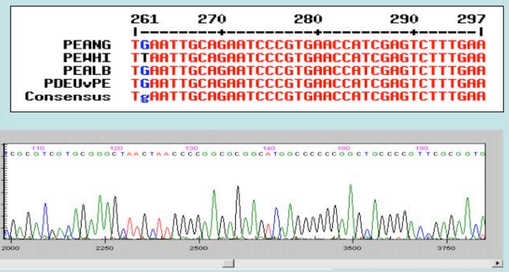
RESULTS / DISCUSSION

Figure 1 displays the phylogeny of Penstemon created using the ITS sequence. On this tree the six *Penstemon* subgenera, as determined by the morphological phylogeny, have been mapped to it. If the morphological division support the molecular tree then the colored sections should be grouped together to show the separate origins of each subgenera, but clearly some subgenera are imbedded in others. For example, Habroanthus branches out from the subgenus *Penstemon*. This suggests this subgenus is derived from the Penstemon subgenus. Subgenus Dasanthera is assembled well together. This suggests that the subgenus Dasanthera division to be a "true" evolutionary branch. However one species from the subgenus Cryptosemon nudges out. This is a monotypic genus, narrowly endemic to only a few locations in Plumas County, California, so this suggests that it should not be included as a separate subgenus, but rather as a variant in the subgenus Dasanthera.

There is a variety of floral forms within the genus *Penstemon*. It is thought that pollinators drive selection for these different forms. So we were interested to see if flower form and color have arisen once and multiple species have radiated from there, or if each has arisen multiple times. Figure 2 has all the red-flowered *Penstemon* used in this study mapped to our tree. From this we see that red flower forms occur in all at different places in the tree, and therefore has arisen multiple times in the history of the genus. This is also true of flower size (Figure 3).

CONCLUSIONS

1)We see that the current divisions of the Penstemon genus may not accurately reflect the evolutionary origin of the species, as some of the smaller subgenera may actually be more accurately included in some of the larger subgenera.
2) Differences in flower size and color have arisen multiple times within the *Penstemon* genus which suggest these characteristics may have played an important role in the extensive radiation within this genera.



METHODS

Polymerase Chain Reaction. Thirteen Penstemon species were taken from the Chicago Botanic Garden field for DNA extractions (P.Caryi, P.Crandalii, P.Deustrus var. Pedicellatus, P.Angustifolius var. Caudatus, P.Grandiflorus, P.Albertinus, P.Hallii, P.Snalli, P.Rostriflorus, P.Calycosus, P.Nitidus, P.Eatoni, and P.Breviculus) the remaining species were taken from GenBank (http://www.ncbi.nlm.nih.gov/). DNA was extracted using a FastPrep Kit. The ITS region was amplified using the ITS2 and ITS5 primers. The PCR product was cleaned and used as a template for sequencing, using the Beckman Coultier sequencing kit. The sequenced product is cleaned through Ethanol precipitation and then loaded into the CEQ 8000 Genetic Analysis System-Sequencing machine. They are covered with a drop of mineral oil to prevent evaporation. The sequences generated are examined on the computer and then aligned to check quality. Misread bases or any other errors are corrected before the sequence is downloaded.

Nexus Preparation. Once the DNA sequences are downloaded-they were copied into MULTALIN (http://prodes.toulouse.inra.fr/multalin/multalin.html) -where the sequences are cut and converted into a FASTA format. This is done so that all sequences have equal digits, along with proper spacing.