

INTRODUCTION

BACKGROUND

The Hawaiian native mints are one of the largest plant diversifications in the Hawaiian Archipelago. This potential adaptive radiation represents a great deal of morphological variation, which is thought to have occurred relatively recently, due to the young age of the Hawaiian Islands, ranging from ca. 0.5 to 5.0 million years old¹. Prior studies indicate that this radiation has descended from within American members of the genus *Stachys*, a large and widespread group of mints commonly known as hedge-nettles².

Significant evidence has pointed to an origin for the Hawaiian native mints involving different polyploid species of *Stachys*². The hybrid origin may explain some of the extensive morphological diversification that has occurred in this group².

The Hawaiian lineage consist of 58 species in 3 genera, *Haplostachys*, *Phyllostegia*, and *Stenogyne* (Fig. 1). They exist in a great variety of habitats, and their extensive morphological variation include flower structures consistent with both bird and insect pollination, dry and fleshy fruits, as well as a great deal of forms, including herbs, vines and sub-shrubs. This morphological variation is accompanied by a great lack of genetic variation, especially when compared to *Stachys*¹.



Fig. 1 – Representatives of the Hawaiian mint genera (from left to right, along the top row) *Haplostachys haplostachys*, *Phyllostegia hispida*, and *Stenogyne rugosa*, and the three groups of *Stachys* that appeared as a result of this analysis (bottom row) *S. elliptica* (yellow), *S. byzantina* (purple) and *S. ciliata* (green).

AIMS AND RATIONALE FOR THIS STUDY

Among the goals of this study is to determine if South American (SA) members of *Stachys* played a role in the hybrid origin of the Hawaiian native mints since prior studies have omitted species from this region. Although providing limited sequence variation within the lineage, chloroplast and ribosomal DNA sequence data suggest that the Hawaiian native mints are the result of a hybridization event involving members in two separate lineages, one of western, temperate North American (NA) and another of Mexican *Stachys*². If SA *Stachys* were to exist within both of these lineages, it would indicate that these mints are the descendants of the same hybridization event that is thought to have resulted in the Hawaiian radiation. If they were only to exist in one of these lineages, it would suggest that they were not involved in this hybridization event.

This study has utilized a putative homologue of the axial regulator, *AFO* (*ABNORMAL FLORAL ORGANS*), a member of a small transcription factor family that has not previously been utilized for phylogenetic analysis³. Preliminary investigation has recognized some sequence divergence³, which may provide utility for the elucidation of phylogenetic relationships through methods employed in this study.

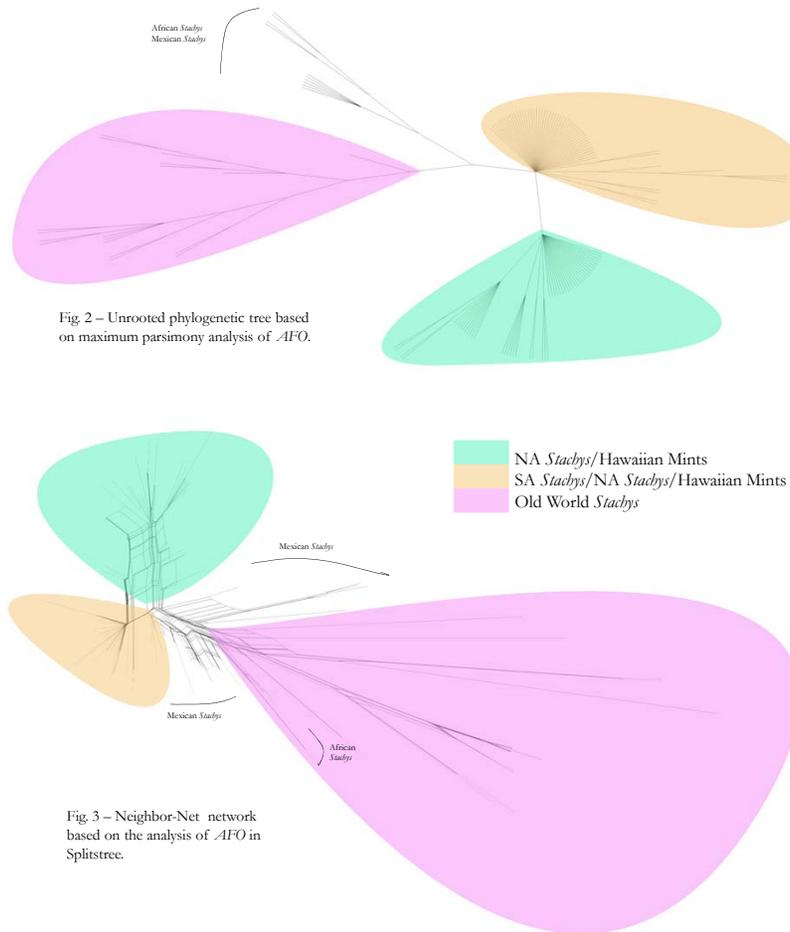


Fig. 2 – Unrooted phylogenetic tree based on maximum parsimony analysis of *AFO*.

Fig. 3 – Neighbor-Net network based on the analysis of *AFO* in Splitstree.

METHODS

AMPLIFICATION, CLONING AND SEQUENCING

Specific primers³ were used to amplify *AFO* in species of *Stachys* and the Hawaiian mints using PCR (polymerase chain reaction). Cloning was done on amplified products to separate allelic variants due to polyploidy. Specimens were sequenced at the Roswell Park DNA Sequencing Core Facility. Sequences were aligned using ClustalW and BioEdit.

PHYLOGENETIC ANALYSES

A variety of phylogenetic inference methods were used to analyze 138 sequences representing 63 species. Among them were (1) a comprehensive maximum parsimony approach with a variety of tree space exploration techniques and bootstrap resampling as implemented in TNT⁴, (2) a maximum likelihood approach with a rapid bootstrap algorithm as implemented in RAxML⁴ (not shown), (3) a Bayesian inference analysis approach in MrBayes 3.1.2⁵ (not shown), and (4) a neighbor-joining approach with bootstrap resampling in SplitsTree 4.11.3⁶ (not shown). Bayesian and maximum likelihood methods used a GTR molecular evolution model as recommended by jModeltest⁷. Additionally, a network analysis using a Neighbor-Net algorithm with LogDet distances in SplitsTree 4.11.3⁶ was performed.

DISCUSSION

ORIGIN OF THE HAWAIIAN MINTS

The different phylogenetic inference methods all resulted in support of the sorting of the Hawaiian mints into two groups alongside different species of New World *Stachys* (Figs. 2-3). One group contains primarily members of *Stachys* from temperate North America with Hawaiian mints (Fig. 2; green) and another contains predominantly South American *Stachys* and Hawaiian mints (Fig. 2; yellow). This study is consistent with previous studies that point to a hybridization event in what is contemporary Southwestern United States prior to the colonization of Hawaii², but also supports an alternative hypothesis that there were two separate colonization events of Hawaii. Chloroplast and ribosomal DNA sequence data, however, support the monophyly of the Hawaiian mints⁸, and it is possible that the presented single-low copy nuclear DNA sequence data exhibit incomplete lineage sorting of alleles and signs of reticulate relationships in a high-polyploid lineage of hybrid origin. Extensive reticulation between *AFO* alleles is also supported by the network analysis (Fig. 3). Furthermore, the limited phylogenetic resolution can also be contributed to very low sequence divergence, which has been shown previously with non-coding ribosomal DNA⁸ as well as another low-copy nuclear gene, *Waxy* (unpublished data), thereby confirming a very recent and rapid divergence of the Hawaiian mints and New World *Stachys* species. Further analysis of other markers is needed to resolve the relationships between the species within the American/Hawaiian lineage of mints.

UTILITY OF *AFO* IN PHYLOGENETIC STUDIES

In particular the non-distance based methods of inference produced trees of low resolution resulting in polytomies consisting of members of the Hawaiian and New World *Stachys* lineage. Consequently, *AFO* as a phylogenetic marker for resolving phylogenetic relationships in this group is of limited utility. However, considerable sequence divergence and phylogenetic resolution appears to occur among Old World *Stachys* species providing promise as a marker for use in phylogenetic reconstruction in this group. It is possible that it could be utilized as a phylogenetic marker in other plant groups.

REFERENCES

- Lindqvist C, Laakkonen L, Albert V: Polytetraploidy variation in a flowering time protein correlates with island age in a Hawaiian plant radiation. *BMC Evolutionary Biology* 2007, 7:105.
- Lindqvist C, Albert VA: Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 2002, 89:1709-1724.
- Lindqvist C, Scheen AG, Yoo MJ, Grey P, Oppenheimer D, Leebens-Mack J, Soltis D, Soltis P, Albert V: An expressed sequence tag (EST) library from developing fruits of an Hawaiian endemic mint (*Stenogyne rugosa*, Lamiaceae): characterization and microsatellite markers. *BMC Plant Biology* 2006, 6(1):16.
- Stamatakis A, Hoover P, Rougemont J: A Rapid Bootstrap Algorithm for the RAxML Webservers. *Systematic Biology* 2008, 57(5): 758-771.
- Huelsenbeck JP, Ronquist F: MrBayes: Bayesian Inference of Phylogenetic Trees. *Bioinformatics* 2001, 17: 754-755.
- Huson DH, Bryant D: Application of Phylogenetic Networks in Evolutionary Studies. *Molecular Biology and Evolution* 2006, 23(2):254-267.
- Posada D: jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 2008, 25: 1253-1256.
- Lindqvist C, Motley TJ, Jeffrey JJ, Albert VA: Cladogenesis and reticulation in the Hawaiian endemic mints (Lamiaceae). *Cladistics* 2003, 19(6):480-495.

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