An American Origin for the Hawaiian Native Mints
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INTRODUCTION

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

The Hawaiian lineage consists 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*.

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*. 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 regulatory gene *AFO* (*ANRA209*), as a probe for a specific region in a transcript from a small number of species in this group, including herbs, vines and sub-shrubs. This morphological variation is accompanied by a great lack of genetic variation, especially when compared to *Stachys*.

METHODS

**AMPLIFICATION, CLONING AND SEQUENCING**

Specific primers 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. Sequences 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 TNT, (2) a maximum likelihood approach with a rapid bootstrap algorithm as implemented in RAxML (not shown), (3) a Bayesian inference analysis approach in MrBayes 3.1.2 (not shown), and (4) a neighbor-joining approach with bootstrap resampling in SplitsTree 4.11.3 (not shown). Bayesian and maximum likelihood methods used a GTR molecular evolution model as recommended by ModelTest. Additionally, a network analysis using a Neighbor-Net algorithm with LogDet distances in SplitsTree 4.11.3 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 Hawaii, 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 mints, 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 DNA as well as another low-copy nuclear gene, *Ifp* (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


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