Identifying genetic differences in *Castilleja sp.* relative to their taxonomy and geographic location

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Introduction

- Traditionally, species are identified by morphology, but sometimes morphological traits vary, and it is harder to ID an individual.
- With advancements in DNA sequencing, another way to identify species is to use DNA barcodes or genetic fingerprints
- It is assumed that all organisms in a species would have a similar genetic fingerprint due to their close-knit genetic makeup.
- For some systems, barcoding isn't an effective identification method when related species are nearby and have flexible species boundaries (this leads to frequent hybridization).
- When species hybridize closely, similar haplotypes should be present in a given range.

Study System

The genus *Castilleja* (also known as the Indian Paintbrushes):

- Have proven difficult to classify taxonomically
- Little is known about their evolutionary tract, it has been a struggle to follow due to a possible rapid divergence and frequent hybridization

This project was focused on studying genetic variations in different populations of four *Castilleja* species (*C. sessiliflora*, as well as *C. lindheimeri, C. purpurea, C. citrina,* which recently were elevated to species status)[2] from the Eastern range of the genus in central and southern US.

The purpose was to be able to identify haplotypes to see if a genetic barcode could be made for the species.

Recent evidence has suggested interspecific gene flow is common in *Castilleja* occurring within a given area (Tank, pers. Comm.), this could indicate geography, rather than species identity is the main driver of gene flow in this group.

Questions:

1. Are the haplotypes related to species identity or just geographic distance?
2. Can barcodes be made for this genus?

C. sessiliflora

C. citrina

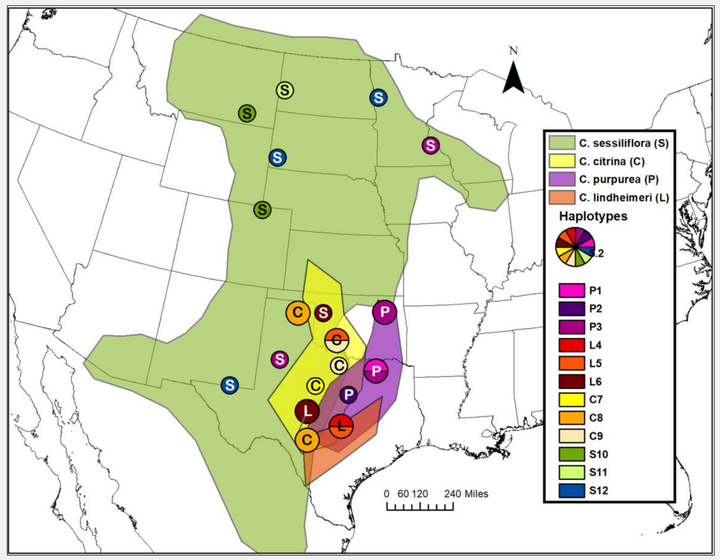

Figure 1. Geographic distribution of chloroplast haplotypes. Colored polygons represent species ranges. Colors of circles represent haplotypes present. Size of circles show number of individuals represented. Letter represents species identified in the field.

C. purpurea

C. lindheimeri

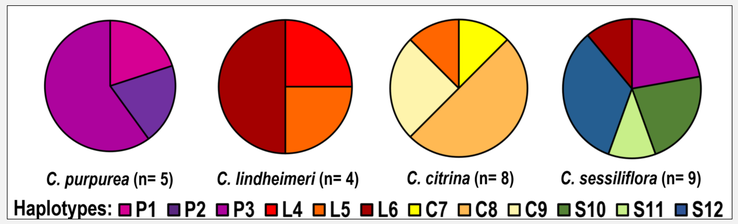

Figure 2. Haplotype proportions by species. Size of wedges show number of individuals with said haplotype per species.

Haplotype	C03			C04	C06		C09	
	60bp	514bp	595bp	220bp	371bp	633bp	230bp	510bp
Consensus	A ...	C ...	-	T	C ...	G	T ...	G
P1	G	T	*	*	*	A	*	*
L4	G	*	*	*	*	A	*	*
C7	G	*	*	*	*	*	*	*
C8	*	T	*	*	*	A	*	*
P2	*	*	T	G	*	A	C	*
C9	*	*	T	*	G	A	*	A
L5	*	*	T	*	*	A	*	*
S10	*	*	T	*	*	*	*	*
L6	*	*	*	G	*	A	C	*
S11	*	*	*	*	G	A	*	*
P3	*	*	*	*	*	A	*	*
S12	*	*	*	*	*	*	*	*

Table 1. Genetic differences in chloroplast sequence by haplotype. Primer and variable regions specified above.

Methods

- CTAB DNA extractions were done with previously collected samples.
- Ten Chloroplast and Ten Nuclear Primers were tested[3,4] and six (C03,C04,C06,C09,N22, and N29) were chosen to amplify the genes being looked at.
- PCR plates were prepared containing the sample DNA with each of the selected primers.
- After, the plates were cleaned using the Exo/Sap Procedure. Followed by Cycle sequencing with v 3.1 Big Dyes.
- The sequence was then cleaned applying the Big Dye precipitation protocol. Once completed, the plates went through sequence analysis using the Sanger Method with the ABI 3730.
- The data received was reviewed, and haplotypes were found with CodonCode Aligner.
- Haplotypes identified were then organized and mapped to look for geographic vs. species/morphological patterns.

Results

The haplotypes identified were demonstrated geographically (Fig.1) and by species (Fig.2). The geographic distribution of the chloroplast haplotypes (Fig.1) shows that no clear indication exists between species individuals and geographic region. Neither does it show haplotypes in response to only one species.

Discussion

- Based off the mapping distribution of the chloroplast haplotypes (Fig.1), it was established that geographic proximity alone does not influence the differences in the genetic sequence between the species, nor is species identity totally responsible either.
- Notably, in two populations with multiple species co-occuring, haplotypes were consistent within species and distinct among species growing in close proximity. This is evidence that species boundaries may be important in structuring gene flow within populations.
- While some haplotypes are specific to a species of *Castilleja*, not all of them are exclusive to just one.
- As a result, it could prove difficult to create genetic fingerprints for this genus.
- More work can be done on studying evolutionary relationships among the haplotypes. Further projects may include sequencing more samples with chloroplast primers, as well as using nuclear primers to investigate hybridization in the genus.

Acknowledgements

I would like to thank Katie Wenzell for giving me the opportunity to work with her and her project. Thank you for your time, patience, and encouragement. I would also like to thank Andrea Kramer, Jeremie Fant, and Hilary Noble for their guidance and constant support. I am eternally grateful. As well as Manal Amjad, for all her help in the lab.

Appreciation to the NSF-REU Grant DBI-1757800 for financial support and to the Chicago Botanic Gardens for allowing usage of their facilities.

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