

Molecular Markers Distinguish Hybridization Patterns in *Castilleja*

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Introductions

Castilleja levisecta (golden paintbrush) is an endangered flowering plant restricted to the Pacific Northwest United States (Figure 1). This species is undergoing restoration in prairies native to another *Castilleja* species, *C. hispida*, which is being used to restore habitat to an endangered butterfly⁵. However, it has been observed that hybridization of the two *Castilleja* species can occur², which could cause the genetic swamping of *C. levisecta*.

Hybridization between the two species can occur on the top and bottom inflorescence. Although *C. levisecta* flowers first, *C. hispida*'s flowering time overlaps with *C. levisecta*'s. Due to flowering phenology, outcrossing between the two can result in hybridization differences between the top and bottom [earlier and later, respectively] inflorescence². It is hypothesized that there will be a distinguishable variance in the microsatellite regions between the two species. Additionally, these variances will allow us to understand the likelihood of hybridization related to the position of the inflorescence.

Materials

1. *Castilleja hispida*:⁴ photo taken by Tom Kaye

- a. Wolf Haven (WH)
- b. Joint Base Lewis-McChord (JBLM)
- c. Scatter Creek South (SCS)
- d. Bald Hill (BH)

2. Assume hybrids [Glacial Heritage]:

- a. Maternal Plants (F1)
- b. Presumed F2

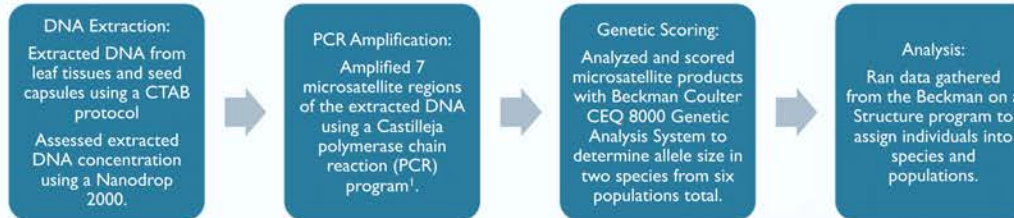
3. *Castilleja levisecta*:⁴ photo taken by Tom Kaye

- a. Ebey's Landing
- b. Fort Lewis
- c. Rocky Point
- d. Naas



Figure 1. Generalized map of wild and reintroduced populations of *C. hispida*, *C. levisecta*, and F1 and F2 hybrids.

Materials/Methods



Results

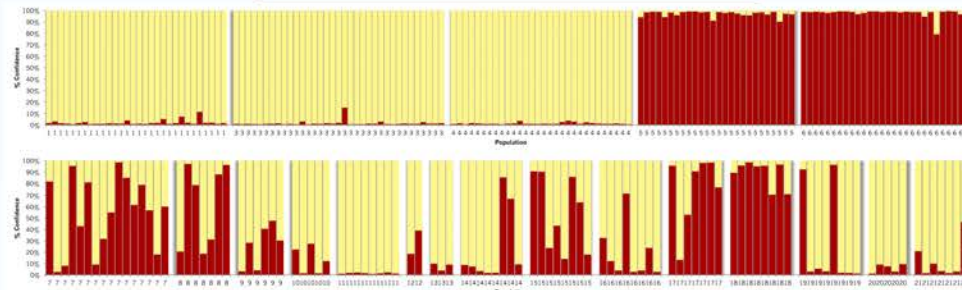


Figure 2. Top: structure analysis for *C. hispida* and *C. levisecta*. Assigned into clusters of $K=2$, yellow corresponds to % confidence of *C. levisecta* and red corresponds to % confidence of *C. hispida*. Bottom: structure analysis for F1 and F2 hybrids. Assigned into clusters of $K=2$, the mix of yellow and red determine the % confidence towards *C. hispida* or *C. levisecta*.

Population	Species	Hybrids	Levisecta	Hispida	Sample ID
1	Levisecta	2%	98%	0%	1
2	Levisecta	2%	98%	0%	2
3	Levisecta	2%	98%	0%	3
4	Levisecta	1%	99%	0%	4
5	Hispida	98%	2%	0%	5
6	Hispida	98%	2%	0%	6
7	F1 hybrid	54%	46%	0%	7
8	F2 hybrid	62%	38%	0%	8
9	F2 hybrid	74%	26%	0%	9
10	F2 hybrid	13%	87%	0%	10
11	F2 hybrid	2%	98%	0%	11
12	F2 hybrid	2%	98%	0%	12
13	F2 hybrid	8%	92%	0%	13
14	F2 hybrid	20%	80%	0%	14
15	F2 hybrid	54%	46%	0%	15
16	F2 hybrid	87%	13%	0%	16
17	F2 hybrid	75%	25%	0%	17
18	F2 hybrid	89%	11%	0%	18
19	F2 hybrid	28%	72%	0%	19
20	F2 hybrid	3%	97%	0%	20
21	F2 hybrid	34%	66%	0%	21

Figure 3. Summary of % confidence, number of individuals, and population for wild and reintroduced *C. hispida*, *C. levisecta*, and F1 and F2 hybrids.

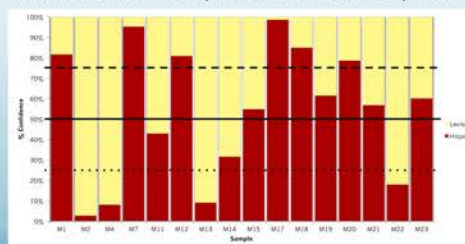


Figure 4. Structure analysis for putative hybrid maternal lines assigned into clusters of $K=2$. A straight black line at 50% represents F1 and dotted line at 75% and 25% represents backcross to *C. hispida* and *C. levisecta*, respectively.

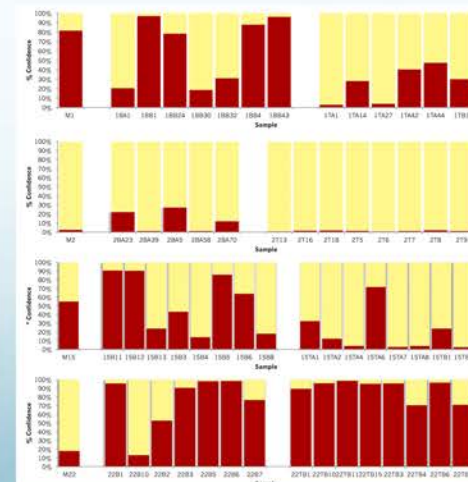


Figure 5. Structure analysis of M1, M2, M15, M22, and their respective offspring. Each graph represents genetic composition of maternal plants followed by the genetic composition of 5-8 seeds collected from the top and bottom capsules.

Discussion

To determine whether *C. levisecta* and *C. hispida* had detectable differences in their microsatellite regions, I ran a Structure analysis on 21 populations. Based on the results, the 21 populations were assigned into two distinct clusters, distinguished as *levisecta* (yellow) and *hispida* (red), respectively. From Figure 2, there are notable differences between the two *Castilleja* species, displaying a confidence over 90% as either *C. levisecta* or *C. hispida*. Comparing these differences to the Structure analysis from population 7 showed that the assumed F1 hybrids from Glacial Heritage were a *levisecta-hispida* mix; however, the analysis also indicated that some of the hybrids were not F1. Assuming the hybrids were all F1, the Structure analysis would display an approximately linear pattern at 50% confidence across the population. Yet, some of the hybrids displayed a 75% confidence towards *levisecta* and others a 25%, suggesting that the hybrids are backcrossing to their pure species (Figure 4). Additionally, these results suggest that not all hybrids are distinguishable.

To determine whether hybrids displayed a noticeable hybridization pattern, I compared the Maternal F1 to the F2. Yet, comparison could only be analyzed for M1, M2, M15, M22 (see Figure 3), and their respective offspring due to missing Maternal F1 samples or polyploidy issues within the F2. However, the Maternal F1 and F2 lines that were analyzed for both the top and bottom inflorescence indicated that a directional hybridization pattern was not observed (Figure 5). For example, M2 and M22 were backcrossing towards *levisecta*. Although the bottom inflorescence for their respective offspring backcrossed towards *hispida*, M2's offspring's top inflorescence backcrossed towards *levisecta*; whereas, M22's offspring's backcrossed towards *hispida*. This implies that there is no clear hybridization pattern. Nevertheless, pollination is random and less specific, therefore, there is no pattern for which inflorescence becomes pollinated by time or position².

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