Investigating putative hybrids of *Fremontodendron decumbens* and *F. californicum* in the Sierra foothills

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Introduction

Fremontodendron (Flannelbush, Fremontia) is a genus of shrubs in the Malvaceae (Mallow family) native primarily to California, and found sporadically in Arizona, and Baja California Norte. There are three species in the genus, *Fremontodendron californicum* is widespread throughout the range and is highly variable in its appearance, *Fremontodendron mexicanum* is endemic to Otay mountain in Southern California, and *Fremontodendron decumbens* is endemic to gabbro soils in the Sierra Nevada foothills. *F. decumbens* is currently only known from several occurrences on and around the Pine Hill Preserve in El Dorado County and was listed as endangered in 1996. *F. decumbens* is mainly distinguished from *F. californicum* by its orange or reddish flowers and decumbent habit. Many plants with a decumbent habit but lacking the floral characters to be placed in *F. decumbens* have been collected over the years, primarily in the Northern Sierra Nevada/Cascade foothills. Because these individuals are morphologically intermediate, there has long been speculation as to whether these could be hybrids between *F. californicum* and *F. decumbens*, and whether they should be appropriately treated as *F. decumbens*, or whether they could be a new species.

The recovery plan for Gabbro Soil Plants of the Sierra Nevada Foothills (2002) recommended that "the decumbent *Fremontodendron* within Yuba and Nevada counties should be secured and protected unless they are determined not to be the listed [*F. decumbens*]" (USFWS, 2002). In 2006, a study of the genetics and morphology of the the decumbent *Fremontodendron* in Yuba and Nevada counties found that they did not group conclusively with either *F. decumbens* or *F. californicum* (Kelman et al., 2006). However, based on the presence of unique alleles in the decumbent populations they genotyped, they suggested continued conservation until further data become available. This study speculated that the decumbent populations in Yuba and Nevada counties are likely to be the product of hybridization between *F. decumbens* and *F. californicum*. Given the importance of determining the genetic status of the decumbent *Fremontodendron* found outside of the Pine Hill area (hereafter referred to as *F. aff. decumbens*), we tested the hypothesis that *F. aff. decumbens* populations are derived from the hybridization of *F. californicum* and *F. decumbens*. If this hypothesis is true, we expect to see either genetic intermediacy of the *F. aff. decumbens* individuals, which would support recent hybridization, or the presence of alleles associated with *F. decumbens*, as can be detected using a variety of population genetic tests.



Figure 1: Map of collection sites. Right panel shows flowers of *F. californicum* (A), *F.* aff. *decumbens* (B) and *F. decumbens* (C)





Each bar represents one individual, the color of the bar represents the proportion of predicted ancestry, and the bars are grouped according to collection site and species hypothesis based off of morphology. K values indicate the number of populations that the model will assign ancestry fractions to. The supervised label indicates a model where known *F. californicum* and *F. decumbens* samples are used as training data. This improves the accuracy of the model, and our conclusions are based off the K=2 supervised results, as well as K = 3&4.

Methods

Sampling

• We collected leaf samples as well as herbarium voucher specimens from 99 individuals across 12 sites in Nevada, Yuba, El Dorado, Napa, Santa Cruz, and Tehama counties (Fig. 1)

DNA extraction and sequencing

- DNA was extracted using either a modified CTAB method, or the NucleoMag Bacteria DNA isolation kit
- Libraries for whole genome sequencing were prepped either at the UC Davis Genome center, or the California Conservation Genomics Project Minicore, using the plexWell library preparation protocol.

Figure 4: PCA of morphological measurements $\overline{\nabla \quad \nabla} \nabla$

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0.4

0.2



• 2x150 paired end sequencing was performed either at the UC Davis Genome center, or the UCLA Minicore facility on an Illumina Hiseq4000 machine

Analyses

- Raw reads were processed by the the California Conservation Genomics Project bioinformatics team, using the SNParcher pipeline under the default settings
- We used genomic principal components analyses as well as an evolutionary clustering method as implemented in ADMIXTURE to investigate population structure and admixture in our 99 individuals
- We measured 10 morphological characters in the field, on 68 individuals, conducted a PCA, and used ANOVA and Tukey's HSD test to determine whether there were any significant difference in character traits. Characters measured were Sepal Color, Sepal Base Color, Peduncle Length, Leaf Length, Petiole, Length, Leaf Width, Leaf Lobes, Trichome Length, Plant Width, Plant Height, and Width Height Ratio.



Conclusions

We found little evidence to suggest that the potential hybrid individuals are in fact derived from recent or ancestral hybridization events. Rather, our data are consistent with the well established view that *F. californicum* is a highly variable species, and suggest that a decumbent habit and/or somewhat darker flowers do not indicate hybrid parentage. Taxonomically, it seems clear that the *F.* aff. *decumbens* individuals, should be incorporated within *F. californicum*.