CONSERVATION GENETICS OF ENDANGERED SUISUN THISTLE AND ITS CONGENERS

CARLY MIRANDA, CANTLEY LAB
OVERVIEW

INTRODUCTION
- Background of Suisun thistle ecological and genomic analysis

HYPOTHESIS
- Potential applications of population genetic structure in Suisun thistle restoration

METHODS
- Fieldwork, wet lab, and computational analysis

RESULTS
- Basic statistics, Wright’s F statistics, and BIC for k-means clustering

IMPACT
- Outplanting and conservation implications
INTRODUCTION

Left: *Cirsium hydrophilum* var. *hydrophilum* (Suisun thistle) in bloom
Center: Suisun thistle basal rosette (CA Rare Plant Rank: 1B.1)
Right: *Cirsium hydrophilum* var. *vaseyi* (Mt. Tamalpais thistle) in bloom
NOT TO BE CONFUSED WITH...

*Cirsium vulgare*

Invasive European Bull Thistle
Spear Thistle
Common Thistle

Allotetraploid $2n = 4x = 64^{[1]}$
Larger flowers, widespread and common in lands distant from its origin.
RUSH RANCH
GEOMORPHIC UNITS

- Subtidal channel beds
- Fringing tidal marsh
- Upper creek banks
- Tidal marsh-terrestrial buffer
URBANIZATION
Historically observed impacts of cattle grazing are thought to be responsible for a population bottleneck event impacting Suisun thistle in the late 20th Century."G2012".
INSECT PREDATION IMPACTS

Left: *Lasioderma haemorrhoidale*
Above: *Rhinocyllus conicus*
INVASIVES PERENNIAL PEPPERWEED IMPACTS
COHABITATING EDAPHIC SPECIALISTS

Rush Ranch Open Space Preserve
Solano County, CA

Endangered Endemics:

- **Symphyotrichum lentum**
  Suisun Marsh Aster (left)

- **Cordylanthus mollis** ssp. **Mollis**
  Soft Bird’s Beak (right)
HISTORICAL BACKGROUND OF CIRSIUM HYDROPHILUM ECOLOGICAL AND GENETIC ANALYSIS

- Phylogeny and ecological radiation of New World thistles based on highly conserved ITS and ETS rDNA sequencing data. (Kelch and Baldwin, 2003)
- Five plots within Suisun Marsh are identified as suitable restoration sites for C. hydrophilum var. hydrophilum. (USFW, 2013)
- Confirms the presence of a CA-FP adaptive radiation clade. (Ackerfield et al., 2020a & 2020b)
- Quantifies seed set and germination in outcrossing and selfing Suisun thistles. Evaluates salinity and inundation tolerance in Suisun thistle seeds. (Schneider and Boyer, 2021)
- Conduct population genetic analysis of C. hydrophilum. Determine if extant Suisun thistle stands comprise one or more populations. (Cantley Lab, 2022)
Map of *Cirsium hydrophilum* collection sites

Mt. Tam thistle

Suisun thistle
Brackish tidal marsh with daily and seasonal variation in salinity and tides.

Salinity varies between oligohaline and mesohaline conditions, averaging 4.7 ppt [Buffington 2020].
PREDICTED DEMES OF SUISUN THISTLE
Our sampling efforts between 2019-2020 were greatly hindered due to restricted lab access on campus and redirected sequencing efforts caused by the COVID-19 pandemic.
METHODS: COMPUTATIONAL ANALYSIS

Remote computational access
- Install Docker containerization for virtual workspace
- Use Windows Powershell to communicate through containers

*De novo* genome assembly
- Sample demultiplexing
- Indexed primers removed

Hard filtering
- Limit missingness
- Minor allele frequency
- Linkage Disequilibrium

Pairwise Fst analysis
- Comparison within Suisun thistle
- Comparison between taxa

$k$-means clustering
- DAPC
- PCA

He, Ho, and Fst evaluated by taxa and assigned deme
- Data transformation for compatibility with R programming software
RESULTS:

BASIC STATISTICS

WRIGHT’S F-STATISTICS

\[ F_{ST} = \frac{(FST - FIS)}{(1 - FIS)} \]

Table 1: Suisun thistle \( H_0 \), \( H_E \) and \( F_{IS} \) by geographic subdivisions

<table>
<thead>
<tr>
<th>Deme (sample size)</th>
<th>Observed Heterozygosity (( H_0 ))</th>
<th>Expected Heterozygosity (( H_E ))</th>
<th>Inbreeding (( F_{IS} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=34)</td>
<td>0.005890805</td>
<td>0.07192816</td>
<td>0.8984</td>
</tr>
<tr>
<td>D (n=10)</td>
<td>0.007655172</td>
<td>0.07051427</td>
<td>0.8506</td>
</tr>
<tr>
<td>T (n=6)</td>
<td>0.003839080</td>
<td>0.04794540</td>
<td>0.8628</td>
</tr>
<tr>
<td>X (n=15)</td>
<td>0.005954023</td>
<td>0.06782759</td>
<td>0.8794</td>
</tr>
<tr>
<td>RR (N=65)</td>
<td>0.006</td>
<td>0.065</td>
<td>0.911</td>
</tr>
</tbody>
</table>
PAIRWISE-$F_{ST}$ VALUES BETWEEN PREDICTED DEMES OF SUISUN THISTLE
EXAMPLE DATASET: BAYESIAN INFORMATION CRITERION (BIC)
Value of BIC versus number of clusters

SUISUN THISTLE BIC TO INTERPRET K-MEANS CLUSTERING
Suisun thistle
Discriminant Analysis
of Principal Components of Variance
CALIFORNIA CONGENERS OF SUISUN THISTLE

*Cirsium hydrophilum* var. *hydrophilum*

Mt. Tamalpais thistle, serpentine dweller

Habitat in the Marin Municipal Water District is restricted to creek banks, vertically sloped roadside disturbances, and boggy marsh areas.
MT. TAMALPAIS COHABITATING SPECIES

Aquilegia formosa
Western Columbine (common)

Blue-green rocky serpentine soil

Aster ssp.

Not pictured:
Arctostaphylos hookeri ssp. Montana (Mt. Tamalpais Manzanita), Ceanothus masonii (Mason’s ceanothus), Eriogonum luteolum var. caninum (Tiburon Buckwheat)[2]
MT. TAM THISTLE COLLECTION SITES

- We sampled *Cirsium hydrophilum* var. *vaseyi* East of Kent Lake and South of Alpine Lake (N=24).
PAIRWISE-$F_{ST}$ VALUES BETWEEN THE VARIETIES
DAPC: MT. TAM THISTLE AND SUISUN THISTLE
CONCLUSIONS

- The sampled range of Suisun thistle in this study belong to one core population with no genetic subdivision by predicted demes.
- Inbreeding is extremely high in this population, with the inbreeding coefficient $F_{IS} = 0.911$.
- Observed heterozygosity is an order of magnitude lower than expected heterozygosity ($H_o = 0.006$, $H_E = 0.065$).
Future sequencing of Suisun thistle will include samples from stands at Peytonia Slough and Hill Slough.
IMPACT ON OUTPLANTING RECOMMENDATIONS

- We suggest that outplanting Suisun thistle sourced from within the sampled area at Rush Ranch should cause no evidently harmful effects on the population.
- Restoration of *Cirsium hydrophilum* var. *hydrophilum* in this range will be beneficial to the population since all sequenced samples belong to the same genetic cluster.
- Further analysis of Suisun thistle from Hill Slough and Peytonia Slough sites will be incorporated to disclose any additional and thus far unaccounted for genetic diversity in the species.
DISCUSSION & QUESTIONS?

Thank you for attending and all your support!

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REFERENCES


Population genomics of island mallow, *Malva assurgentiflora* (Malvaceae)
Outline

• Introduction to the island mallows
• Recent taxonomic changes
• Population genomics across *Malva assurgentiflora*
• Population genomics within the northern California Channel Islands subsp. *assurgentiflora*
• Summary and Conservation implications
Phylogenetics of island mallows

Figure 3. BEAST2 chronogram of *Malva* using nrITS data with fossil calibration from Villa-Machio et al. (2018) for the node indicated with circle. Numbers on branches represent Bayesian posterior probabilities. Axis below represents time in MYA.
M. assurgentiflora  M. pacifica  M. lindsayi  M. occidentalis

Where is that subsp. glabra from?
Previous population genetics work (Guilliams et al. 2018)

Northern ChI

Santa Catalina Island

San Clemente Island

Possible admixture?
Smooth petal apex, dull leaves
subsp. *assurgeniflora*

Star-shaped hairs

“Chewed up” petal apex, shiny leaves
subsp. *gabra*

No hairs

Guilliams 2019
Malva in cultivation

subsp. glabra

M. pacifica

subsp. assurgentiflora

x M. pacifica

subsp. assurgentiflora

M. occidentalis
Observation data for *Malva assurgentiflora*

October 2022 (inaturalist.org)
Study Questions

1. Does genomic data continue to support taxonomic circumscription of northern island plants as differing from southern island plants?

2. Does this genomic data support the taxonomic recognition of naturally occurring plants from San Clemente as differing from those on Santa Catalina?

3. With a broad sampling of plants that are morphologically consistent with subsp. assurgentiflora, can we identify the genetic provenance of plants in horticultural and presumed planted settings on the mainland and across the archipelago?

4. Are there unique genotypes in horticultural settings that might be of value in future conservation efforts?

5. What is the within-island and between island genomic diversity of remaining plants on San Miguel and Anacapa?

6. Where are the highest value seedbanking targets across the native range of subsp. assurgentiflora?
Naturally occurring plants

• subsp. *assurgentiflora* from San Miguel and Anacapa islands

• subsp. *gabra* from Santa Catalina and San Clemente islands
Locations of *Malva assurgentiflora* on the Channel Islands

- Location of *M. assurgentiflora* ssp. *assurgentiflora* (Specimens recorded by SBBG)
- Location of *M. assurgentiflora* ssp. *assurgentiflora* (Specimen data from CCH2)
Locations of *Malva assurgentiflora* on San Miguel

- Location of *M. assurgentiflora* ssp. *assurgentiflora* seedlings (Specimens recorded by SBBG)
- Location of *M. assurgentiflora* ssp. *assurgentiflora* (Specimens recorded by SBBG)
- Location of *M. assurgentiflora* ssp. *assurgentiflora* (Specimen data from CCH2)
Dataset assembly and SNP calling

- ddRAD libraries (Trip et al. 2017)
- Dataset assembly (IPYRAD)
- Population genomics statistics (POPULATIONS)
- Principal Coordinates Analysis (DARTR)
- Unrooted phylogeny (RaxML)
- Bayesian population detection/assignment (STRUCTURE in IPYRAD Jupyter)
## Results: summary statistics for each island

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>$N$</th>
<th>$n$</th>
<th>Private</th>
<th>$P$</th>
<th>$Ho$</th>
<th>$He$</th>
<th>$Pi$</th>
<th>$Fis$</th>
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</thead>
<tbody>
<tr>
<td>San Miguel Island</td>
<td>55.32561</td>
<td>2561</td>
<td>0.95432</td>
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<td>0.06189</td>
<td>0.0626</td>
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<tr>
<td>Santa Rosa Island</td>
<td>1.74075</td>
<td>410</td>
<td>0.97245</td>
<td>0.04217</td>
<td>0.03178</td>
<td>0.04672</td>
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<td>Santa Cruz Island</td>
<td>3.8183</td>
<td>82</td>
<td>0.98586</td>
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<td>0.0184</td>
<td>0.02296</td>
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<td>Anacapa Island</td>
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<td>0.0174</td>
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<td>San Nicolas Island</td>
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<td>0.98302</td>
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<td>Santa Catalina Island</td>
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<td>3243</td>
<td>0.88915</td>
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<td>0.1507</td>
<td>0.16699</td>
<td>0.11005</td>
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<tr>
<td>San Clemente Island</td>
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<td>3907</td>
<td>0.88394</td>
<td>0.15164</td>
<td>0.14884</td>
<td>0.18181</td>
<td>0.05935</td>
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</table>
Results: genetic differentiation coefficient ($Fst$) between island sampling locations

<table>
<thead>
<tr>
<th></th>
<th>AI</th>
<th>SC</th>
<th>SMI</th>
<th>SNI</th>
<th>SRI</th>
<th>SCa</th>
<th>M</th>
<th>SCI</th>
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<tbody>
<tr>
<td>AI</td>
<td>0.149321</td>
<td>0.285677</td>
<td>0.18442</td>
<td>0.551654</td>
<td>0.376465</td>
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<td>0.250552</td>
<td>0.628835</td>
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<td>0.435666</td>
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<td>SMI</td>
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<td>0.268628</td>
<td>0.193848</td>
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<td>SNI</td>
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<td></td>
<td>0.60853</td>
<td>0.313416</td>
<td>0.413308</td>
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<td>SRI</td>
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<td></td>
<td>0.267552</td>
<td>0.693318</td>
<td>0.343678</td>
<td></td>
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<tr>
<td>SCa</td>
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<td></td>
<td></td>
<td>0.216809</td>
<td>0.224818</td>
<td></td>
<td></td>
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<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.330006</td>
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</table>
Results: scatterplot of PCo2 vs. PCo1
Results:
unrooted RAxML
Results: structure

<table>
<thead>
<tr>
<th>Nreps</th>
<th>lnPK</th>
<th>lnPPK</th>
<th>deltaK</th>
<th>estLnProbMean</th>
<th>estLnProbStdev</th>
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<td>1</td>
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<td>0.00</td>
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<td>1464.130</td>
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<td>22166.44</td>
<td>8.889</td>
<td>96105.73</td>
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<td>24457.75</td>
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<td>60964.59</td>
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<td>10</td>
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<td>0.286</td>
<td>-113727.25</td>
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<td>7</td>
<td>10</td>
<td>-30962.90</td>
<td>86576.84</td>
<td>0.985</td>
<td>-143790.15</td>
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<tr>
<td>8</td>
<td>10</td>
<td>-119639.74</td>
<td>138548.32</td>
<td>0.609</td>
<td>-263429.89</td>
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<tr>
<td>9</td>
<td>10</td>
<td>18906.58</td>
<td>7120.02</td>
<td>0.030</td>
<td>-244523.31</td>
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<td>10</td>
<td>10</td>
<td>11786.56</td>
<td>0.00</td>
<td>0.000</td>
<td>-232736.75</td>
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</tbody>
</table>
Results: structure
Results: structure
Results: structure

Anacapa & Santa Cruz

San Miguel

San Clemente & Santa Catalina
Results: structure
Results: PCoA northern island mallow (subsp. assurgentiflora)
Results: structure $K=3$
Results: structure $K=3$
Results: structure K=3
So what does it mean?

• Continued support for N/S split
• Little evidence of genetic exchange between islands
• Each island treated independently
So what does it mean?

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• Little evidence of genetic exchange between islands
• Each island treated independently
So what does it mean?

subsp. *glabra* on Clemente:

- New taxon should be recognized from San Clemente
- Additional morphological study might clarify differences between island taxa
- Surveys and tagging of naturally occurring plants
- Out planting should come from seeds sources in original populations on SCl
- Remove any plants of question province
- VERY RARE!
So what does it mean?

Subsp *glabra* on Clemente:

- New taxon should be recognized from San Clemente
- Additional morphological study might clarify differences between island taxa
- Surveys and tagging of naturally occurring plants
- Out planting should come from seeds sources in original populations on SCl
- Remove any plants of question province
- VERY RARE!
So what does it mean?

Subsp. *glabra* on Santa Catalina

- This taxon is also extremely rare!
- Remove any plants both in town and back country consistent with subsp. *assurgentiflora*
- Seedbank plants from Bird and Indian Rocks
- Outplant from those seed sources
So what does it mean?

Subsp. *glabra* on Santa Catalina

- This taxon is also extremely rare!
- Remove any plants both in town and back country consistent with subsp. *assurgentiflora*
- Seedbank plants from Bird and Indian Rocks
- Outplant from those seed sources
So what does it mean?

Northern Channel Islands

• This taxon is also extremely rare throughout its natural range
• Big genetic split between AI and SMI
So what does it mean?

Northern Channel Islands

- This taxon is also extremely rare throughout its natural range
- Big genetic split between AI and SMI
So what does it mean?

Northern Channel Islands
  • Anacapa genotypes are also in all hort samples tested
  • Anacapa genotypes found on SNI and SCr
  • Potential enhancement of genetic diversity of NPS out plantings from accessions at botanic gardens
So what does it mean?

subsp. *assurgentiflora* on Santa Rosa

- contains unique genetics!
- seedbanking has already occurred
- continue to monitor the plants on SRI
- consider planting more widely in restoration activities
So what does it mean?

subsp. *assurgentiflora* on San Miguel

- These genotypes currently not present in the horticulture trade.
- All current growing patches should be seed banked
- Efforts should be made to keep accessions *ex-situ*
Conclusions

• Population genomics is an important first tool to inform conservation of rare plants

• Reveals highly structured genetic diversity and undescribed taxonomic units (prompts closer look at morphology.)

• Life history of *Malva* lends it to being a potential conservation success story across all islands as with ongoing recovery efforts
Acknowledgements

• We thank our island partners at the U.S. Navy, National Park Service, The Nature Conservancy, the Catalina Island Conservancy, US Geological Survey for support in the field and access to plants.

• National Park Service for funding this project under CESU: and partnership with California Institute of Environmental Studies, specifically Mike Parker and Andrew Yamagiwa for partnership and collaboration on this ambitious project.

• Amy Catalano, Sarah Chaney, Robert Delong, William Hoyer, Steve Junak, Denise Knapp, John Knapp, Julia Lambert, Betty Lee, Annie Little, David Mazurkiewicz, Kathryn McEachern, Ken Niessen, Katrina Olthof, Heather Schneider, Robyn Shea, Dieter Wilken, Ian Williams, Cameron Williams
Genetic diversity of the rare and endangered vernal-pool grasses *Orcuttia, Tuctoria* and *Neostapfia* — preliminary findings from population-level sampling and genomic sequencing (ddRADseq)

R. Douglas Stone*, Bryana Olmeda & J. Travis Columbus

*dstone@cnps.org*
Members of subtribe Orcuttiinae and their current status

<table>
<thead>
<tr>
<th>Species</th>
<th>CNPS Rare Plant Inventory</th>
<th>Federal ESA</th>
<th>State CESA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tuctoria fragilis</em></td>
<td>None (Baja endemic)</td>
<td>None (Baja endemic)</td>
<td>None (Baja endemic)</td>
</tr>
<tr>
<td><em>Tuctoria greenei</em></td>
<td>1B.1</td>
<td>Endangered (1997)</td>
<td>Rare (1979)</td>
</tr>
</tbody>
</table>
Neostapfia colusana
Tuctoria mucronata
Orcuttia viscida
Seven species are endemics or near-endemics of California’s Central Valley.

- *Orcuttia californica* occurs in coastal southern California and adjacent Baja California (with isolated stations further south).

- *Tuctoria fragilis* is known from a single locality in Baja California Sur.
No, “Vernal Pools” did not play third base for the Orioles.
Neostapfia colusana seedlings
Habitat for *Neostapfia colusana* (near Turlock Reservoir, Stanislaus County, July 1987)
Habitat for *Orcuttia californica* (near Santa Clarita, Los Angeles County, June 1996)
A large, pure stand of *Neostapfia colusana*
(Hickman vernal pools, Stanislaus County, August 1986)
Study objectives and sampling

• **To test whether the currently recognized genera** (*Orcuttia, Neostapfia, Tuctoria*) **are natural groups, i.e., monophyletic.**
  
  • We sampled all three genera and nine recognized species throughout their range in California and Baja California.

• **To examine the limits of currently recognized species and partitioning of genetic diversity within and between populations.**
  
  • All species were represented by multiple population samples (except *T. mucronata* & *T. fragilis*).
  
  • Population samples were represented by multiple individuals (except *Neostapfia, T. fragilis & O. pilosa*).
Restriction-site Associate DNA Sequencing (RADseq)

- Genomic DNA
- Restriction Digestion
- Size Selection
- Filtering

Population A:
- Locus 1
- Locus 2

Population B:
- Locus 1
- Locus 2

SNP Site
Restriction Site
The dynamic trio a.k.a “Team Awesome” in Dec. 2021
Mr. Orcutt meets Big Data

- 56 samples $\rightarrow$ total of 97 million DNA sequences (average of 1.7 million sequences per sample)

- 10,000+ shared loci across half of our samples (1,000+ loci in all but a few)

- 9 million sites in multiple sequence alignment

- 537,103 single-nucleotide polymorphisms (SNPs)
Results: *Orcuttia* clade maximum-likelihood IQ-TREE

- Neostapfia
- Tuctoria mucronata
- T. greenei
- T. fragilis
- *Orcuttia inaequalis* clade
  - O. pilosa
  - O. californica
  - O. viscida
  - O. tenuis clade
Results: *Orcuttia inaequalis* clade

- 2857_2 Solano
- 2857_9 Solano
- 5725 Tulare
- 5732 Madera
Orcuttia inaequalis
• herbage gray-green
• inflorescences capitate
• caryopsis 1.3–1.5 mm long

Solano population
• herbage green
• inflorescences not capitate
• caryopsis 2 mm long
Results: *Orcuttia tenuis* clade

- 5734 Sacramento
- 2863_3
- 2863_1 Redding
- 2863_2
- 5738 Red Bluff
- 2867_3
- 2867_1 Burney
- 2867_5
- 2868_8
- 2868_4 Timbered Crater
- 2868_7
- 2869_3
- 2869_4 Modoc
- 2869_9
**Orcuttia tenuis**
- branched above base
- inflorescences not crowded

Sacramento population
- branched only at base
- inflorescences congested toward apex
- previously confused with *O. pilosa*
Results: Orcuttia californica

5717 Baja (Vizcaino Desert)
19259B
19259A Los Angeles
19259C

C7
C5 MCAS Miramar
C6
L3
L1 MCAS Miramar
L5
Results: *Tuctoria greenei*

- 5728 Merced
- 5737 Tehama
- 2866_2
- 2866_1 Modoc
- 2866_4
- 2865_7
- 2865_1 Modoc
- 2865_8
Summary of findings

• **ddRADseq data** have resolving power from deepest parts of the phylogeny to the tips (at & below the species level).

• *Tuctoria* as defined by Reeder (1982) is confirmed to be paraphyletic, with some of its members needing to be reclassified.

• *Orcuttia* populations from Solano & Sacramento counties are genetically & morphologically distinct and should be named.

• Evidence of northward range expansion on an evolutionary time-scale (*Orcuttia tenuis, O. californica, Tuctoria greenei*).
Dr. Roy Buck
(1952 – 2019)

The frontier is still here.
Acknowledgments

• **Permitting agencies** (sampling done under permits TE009018-5 issued by USFWS and 2081(a)-16-024-RP issued by CDFW).

• **Staff & students of California Botanic Garden** (Naomi Fraga, Joy England, Cheryl Birker, Carrie Kiel, Courtney Matzke).

• **Other contributors** (Chuck Black, Francesca Cannizzo, Andrea Craig, Malia Forbert, Steve Foreman, Scott Larson, David Muth, David Nicholas, Erik Roen, John Rebman, Darwin Stone, Carol Witham).

• And thanks to YOU for your kind interest & attention!
Unexpected genetic structure and demographic history of a rare redwood forest specialist (Pedicularis dudleyi)

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\textsuperscript{3} Center for Natural Lands Management, Temecula, CA
\textsuperscript{4} San Jose State University Department of Biology
Consequences of rarity

- Increased susceptibility to extinction due to demographic and environmental stochasticity
- Increased risk of inbreeding depression
- Reduced survival and fecundity
- Limited adaptability to changing environments
Causes of rarity

• Environmental change
• Habitat loss
• Novel pathogens
• Competition with invasive species

*Torreya taxifolia*: Endangered by fungal blight and habitat destruction
Causes of rarity

- Habitat specialization
- Metapopulation dynamics and life history traits
- Young evolutionary age

Rare serpentine endemic wildflower, *Calochortus obispoensis*. Photo credit: David Wolfson
Understanding the causes of rarity can inform management

• Historically or innately rare plants may be less susceptible to risks compared to rare species that were more recently widespread

• Understanding the causes of rarity can inform effective management and restoration programs.
Pedicularis dudleyi: Biology

- Orobanchaceae
- Hemiparasitic
- Primarily outcrossing
- Passive seed dispersal
- Reproductive maturity reached at 5 – 6 years and individuals can live for up to 50 years.
**Pedicularis dudleyi: Biology**

- Orobanchaceae
- Hemiparasitic
- Primarily outcrossing
- Passive seed dispersal
- Reproductive maturity reached at 5 – 6 years and individuals can live for up to 50 years.
Pedicularis dudleyi: Conservation status

- Endemic to the redwood forests of Central California
- Listed as rare under the California Endangered Species Act.
Until recently the species was known from three extant populations.
Pedicularis rigginseiae

- In 2019 the southernmost population was described as a novel species based on morphology and ecology.

Photo credit: Rhonda Riggins from Keil, D.J. 2019.
Pedicularis dudleyi: Past distribution

- Two herbarium specimens, one from 1884 and another from 1893 could represent populations that once occurred between the two extant populations.
- 1893, “By road in redwoods San Lorenzo River”
- 1884, “Aptos, Santa Cruz, County, California”
Pedicularis dudleyi: Past distribution

- The Forest of Nisene Marks State Park, just East of Aptos, was clear cut between 1883 and 1923.
- *P. dudleyi* may have once been more widespread and declined due to logging in the early 20th century.
Aims

• Test the morphological and ecological hypothesis that *Pedicularis rigginsiae* is distinct from *P. dudleyi*.

• Describe the genetic diversity and population structure of *P. dudleyi*

• Test the hypothesis that *P. dudleyi* underwent a bottleneck corresponding with increased logging of redwood forests in the early twentieth century
Aims

• Test the morphological and ecological hypothesis that *Pedicularis rigginsiae* is distinct from *P. dudleyi*.
  
  • *ITS and matK–5 ’trnK intron*

  • Six *P. dudleyi* individuals from Portola and Little Sur

  • Two *P. rigginsiae* individuals from herbarium specimens

  • Closely related *Pedicularis* species using sequences from GenBank
Pedicularis rigginsiae

- P. centranthera
- P. semibarbata
  - P. rigginsiae (Rigi)
    - P. rigginsiae (Rig)
      - P. densiflora 2
        - P. densiflora 1
          - P. dudleyi (E2)
          - P. dudleyi (11)
          - P. dudleyi (B5)
          - P. dudleyi (M2)
            - P. dudleyi (60)

Tree scale: 0.01
Aims

• Describe the genetic diversity and population structure of *P. dudleyi*
  
  • ddRADseq
  
  • Restriction enzymes: EcoRI and SphI-HF
  
  • 2,026 variant loci
  
  • 118 individuals in total: Portola N=57, Little Sur N=61
Population structure

K=2

A B
Population structure

<table>
<thead>
<tr>
<th>Population</th>
<th>Little Sur 2</th>
<th>Little Sur 1</th>
<th>Portola</th>
</tr>
</thead>
<tbody>
<tr>
<td>K = 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K = 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Population structure

Direction of river

N

500 m

A.

B.
## Population structure

### Pairwise $F_{ST}$

<table>
<thead>
<tr>
<th></th>
<th>Portola</th>
<th>Little Sur 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portola</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little Sur 1</td>
<td>0.903</td>
<td></td>
</tr>
<tr>
<td>Little Sur 2</td>
<td>0.891</td>
<td>0.348</td>
</tr>
</tbody>
</table>
The Portola population is genetically distinct from the Little Sur populations.
## Genetic diversity within populations

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Private Alleles</th>
<th>% Polymorphic Loci</th>
<th>Ho</th>
<th>He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portola</td>
<td>57</td>
<td>709</td>
<td>28.74</td>
<td>0.021</td>
<td>0.017</td>
</tr>
<tr>
<td>Little Sur 1</td>
<td>33</td>
<td>242</td>
<td>48.06</td>
<td>0.050</td>
<td>0.110</td>
</tr>
<tr>
<td>Little Sur 2</td>
<td>28</td>
<td>248</td>
<td>46.84</td>
<td>0.044</td>
<td>0.121</td>
</tr>
</tbody>
</table>

The Portola population is less genetically diverse than the Little Sur populations.
Aims

- Test the hypothesis that *P. dudleyi* underwent a bottleneck corresponding with increased logging of redwood forests in the early twentieth century
  - Demographic modeling using DIYABC Random Forest
Recent vs. Historic Rarity

Scenario 1
Stable Population Size

Scenario 2
Recent Bottleneck

Scenario 3
Ancient Bottleneck

Portola Little Sur 1 Little Sur 2
Portola Little Sur 1 Little Sur 2
Portola Little Sur 1 Little Sur 2
Recent vs. Historic Rarity

- Preliminary DIYABC RF analysis strongly supports a recent bottleneck scenario (PP = 1.00)
Conclusions

• *Pedicularis rigginsiae* as distinct from *P. dudleyi*, increasing the conservation priority of both species.

• Portola Redwoods and Little Sur populations of *P. dudleyi* are highly differentiated

• There is genetic structuring in upstream and downstream portions of the Little Sur population

• Demographic modeling supports a scenario where the contemporary rarity of the species is explained by a recent bottleneck.
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Lydia Smith

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LEPECHINIA ROSSII
S. BOYD & O. MISTRETTA
(ROSS’S PITCHER SAGE)
CONSERVATION GENOMICS STUDY – ROBERT COMITO, CARRIE KIEL PHD, BILLY SALE, ANTHONY PEREZ, & NAOMI FRAGA PHD

Claremont Graduate University
• **Lepechinia** (ca. 42 species)
• Aromatic herbs and shrubs
• 31 sampled in 2013 study focused on South American spp.
• Only 1 CA sp. sampled
• **L. rossii** not sampled

(Drew and Sytsma 2013)
**Lepechinia rossii**
*Ross' Pitcher Sage*

**Conservation Status**

- **G1**: Critically Imperiled
- **GX**: Presumed Extinct
- **GH**: Possibly Extinct
- **G2**: Imperiled
- **G3**: Vulnerable
- **G4**: Apparently Secure
- **G5**: Secure
- **No Status Rank**

**Rarity Status**

- **California Rare Plant Rank**: 1B.2
  - Rare or endangered in California and elsewhere
  - .2: Fairly endangered in California

- **Federal Listing Status**: Not Listed
- **State Listing Status**: Not Listed

- **State Rank**: S1
  - S1: Critically Imperiled.

- **Global Rank**: G1
  - G1: Critically Imperiled.
DISTRIBUTION AND ABUNDANCE

Los Padres National Forest (LPNF)

Map: N. Fraga
Objectives

Goal: Gather and update baseline information on *Lepechinia rossii*

1. Survey known occurrences to update population size, status, and threats
2. Conduct population genomic analyses using ddRADseq to provide baseline genetic diversity data
3. Collect seeds and vegetative cuttings and develop propagation protocols
4. Integrate the population survey, genomic, and horticultural information to develop a set of restoration and management recommendations
Population genetics methods and analyses

- 132 L. rossii (49 LPNF, 83 ANF)
- ddRADSeq - Maximum Likelihood Phylogeny
- Population Genomics
  - *structure* plot
  - principal coordinate analysis (PCoA)
    - visualize genetic differentiation of populations in multivariate space
- Analysis of Molecular Variance (AMOVA)
  - Using *poppr* package in R - variance between populations
Propagation Trials

- Conducted by Billy Sale (Restoration Project Manager) and Anthony Perez (Restoration Plant Propagator)
- *Lepechinia rossii* can be propagated by both vegetative cuttings and seeds
- Vegetative cuttings more reliable
- Seeds propagation trials mostly resulted in low germination (below 40%)
- Highest germination (above 40%) included some combination of:
  - clipping the seed coat, 24-hour water soak, cold stratification, smoke water added weekly, gibberellic acid
Field Surveys
Population Surveys

- LPNF (EO1) 150–300 individuals
  - patchily distributed, variety of microhabitats
- ANF listed as 2 separate occurrences in CNDDB (2022), but forms one continuous population (ca. 210–270 individuals)
  - along FS Road 6N24 in Ruby Canyon
  - habitat is roadside, in chaparral, and more or less homogeneous.
- Difficult to assess population trends
- Threats: Climate change, long term drought, changing fire regimes, competition from invasive plant species, fuels treatment and road maintenance
IQtree ML Phylogeny
1000 ultrafast MLBS;
6 taxa, 93 accessions;
74,898 loci, 54% missing data
**L. rossii** (Ventura Co.)

**L. rossii** (Los Angeles Co.)

**Outgroups**

IQtree ML Phylogeny
1000 ultrafast MLBS;
6 taxa, 93 accessions;
74,898 loci, 54% missing data
Population Structure

- Alignment of 19,813 loci
- 87 samples, 5.2% missing data
- At K=2, the two populations are clearly defined
- Mean probabilities and delta K values suggest the optimum K=3
- As K increases from 3 to 5, we see genetic groups appear within the LPNF
- More sampling within the LPNF may uncover additional subpopulation structure
Population Genomics Conclusions

• ANF and LPNF are highly genetically differentiated ($\Phi_{st} = 0.28$)
  • little sign of genetic exchange between populations
  • separate considerations regarding their management/restoration
• The ANF population is genetically cohesive lacking variation
  • CNDDB ANF (EO2 & EO3) should be merged into a single occurrence
• Possible high levels of clonality in the population?
  • Further investigation and analyses
• Seed collections in the LPNF needed to capture the full range of genetic variation across the species
Future Directions

- Additional sampling from LPNF (EO1) for better understanding of genetic diversity of *L. rossii*.
- Processing 40 LPNF & 10 ANF for sequencing
- Incorporated with the existing dataset, used in further genomic analyses
- Future results will be reported to the ANF and LPNF for their management consideration
- Additional 77 samples of wild collected *L. fragrans* to be added from two populations in ANF to provide additional context
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• ANF, LPNF, Hopper Mountain NWR
CNPS 2022 Conference
ROOTING TOGETHER