

Surveys, Curation, Cytological and Population Genetic Analysis of 6 Rare Plant Species, within the San Diego MSPA to Inform Establishment of New Occurrences, Population Enhancement, Seed Banking, and Long-term Monitoring

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Overview and Objectives

The Management Strategic Plan (MSP) for western San Diego County (MSP, Vol. 1 App. Table 1D; Vol. 2, Sections 2.1-2.3) has identified several rare plant species within the plan area for which management activities such as enhancing population sizes or establishing new occurrences are ongoing or recommended based on low numbers of individuals and/or low numbers of occurrences or high risk of threat (See Task 7 Table 1). However, the genetic compatibility and genetic structure of these species are largely unknown, and the MSP identifies conducting genetic surveys as a research need to inform development of Implementation Plans, planned translocations, seed banking and other management actions (Table 1). Depending on the results of the genetic analyses, common garden or reciprocal transplant studies may be recommended to measure fitness consequences of potential management actions that need to be determined prior to initiation of management actions such as seed bulking, establishing new populations, or combining seed from more than one source population to augment genetic diversity.

Genetic factors, including ploidy and levels of genetic relatedness among individuals, can influence compatibility, adaptability and survivorship of transplanted individuals or outcrossed offspring. The key genetic concern when faced with restoration choices is determining the appropriate balance between retaining local adaptation (if it exists) and maximizing overall diversity to avoid negative inbreeding effects and retain the ability to respond to future environmental change. Understanding population genetic structure can aid in this assessment by estimating the scale over which adaptation may occur (e.g., what constitutes a local population genetically distinct from others?) and providing information on the distribution of genetic diversity across individuals and occurrences. In addition, genomic studies (the study of 1000s of gene regions) can identify and survey genes that either code for or are closely linked to adaptive traits. An additional goal is to provide a baseline estimate of genetic diversity within these species for monitoring diversity trends over time in response to future disturbances or restoration actions.

The first phase (years 1-2) of this research task will focus on genetic and cytological screening to determine potential ploidy and population genetic differences among occurrences within species. Upon completion, we will convene an expert panel to review results of the genetic studies and then develop specific recommendations for each species relative to the MSP management objectives planned for that species. These recommendations may include designing appropriate common garden or reciprocal transplant studies to determine the fitness consequences of using seed from different populations to increase population size or establish new occurrences. The recommendations will also address MSP objectives involving seed banking and seed bulking needs for each species. The expert panel will also make recommendations on genetic management of populations, including whether genetic connectivity needs to be enhanced or restored to maintain or increase genetic diversity. Recommended and approved studies will be added in the second phase (beginning in year 3). The following questions will be specifically addressed in phase 1:

1. What is the status of documented occurrences?
2. Is there evidence of mixed ploidy levels among or within occurrences?
3. What is current genetic structure among and within occurrences in the MSPA? How vulnerable are the occurrences to genetic drift & loss of genetic diversity and is there gene flow between occurrences?

4. Are there signatures of genetic bottlenecks or lower genetic diversity in populations that have undergone recent reductions due to fire, drought, or other causes, or evidence of local adaptation?
5. Based on the cytological and genetic analysis, what are the recommendations for common garden and reciprocal transplantations, for collecting, bulking and distributing seeds for enhancing existing occurrences, and for establishing new occurrences?

Methods

The first goals of this task are to verify and sample species occurrences, curate and database samples and voucher specimens in the SDNHM Herbarium Database. Currently, verification and voucher specimens are lacking from many occurrences of the six target species across San Diego County. SDNHM will attempt to visit and sample all known occurrences within the Management Strategic Plan Area (MSPA) for six listed species (Table 1). A list of 90 specific locations on Conserved Lands has been compiled for the 6 rare plant species within the MSPA. SDNHM botanists will obtain permission from managing agencies to visit sites. SDNHM botanists will visit, verify locations and sample up to 20 individual plants from each occurrence. Sampling will be non-destructive (with the exception of one voucher specimen from each site). Five to ten leaves or leaf punches will be removed from each of 5-20 individuals per occurrence and placed in a cellophane envelope for dry storage. Specimens will be verified, curated and databased at the San Diego Natural History Museum and be made available for cytological, genetic and morphological research as outlined in the Management Strategic Plan.

Ploidy differences can confer reproductive incompatibility, or hybridization that may have positive or negative survivorship impacts for both parent populations and hybrid offspring populations. USGS will estimate ploidy number using flow cytometry to determine whether (and where) ploidy differences and hybridization may exist within species. USGS will gather data from 2-5 individuals/occurrence/species. Flow cytometry will be performed in the USDA forest service laboratory in Provo, UT, following established protocols.

Next-generation sequencing provides new tools to gather large amounts of DNA sequence data for non-model organisms and to robustly quantify gene flow among occurrences and identify loci that may contribute to local adaptation. A class of approaches useful for landscape-level studies is reduced-representation sequencing, such as RAD-Seq or genotyping by sequencing (GBS). With these approaches, it is now possible to survey thousands of variable regions of DNA, in the form of single-nucleotide polymorphisms (SNPs), at similar costs as using tools that would yield only 20 regions just five to ten years ago.

By analyzing these thousands of SNPs, population genomic diversity can be estimated. In addition, a major advantage of large SNP data sets is the ability to identify SNPs or genes that are under selection. One approach is identifying SNPs that are especially differentiated among populations, or associated to specific site or climatic variables relative to other SNPs. Application of these methods may allow us to identify local adaptive variation.

DNA will be extracted from leaves using standard CTAB protocol or plant extraction kits to generate a large quantity of high quality DNA. We will use an adapted RAD-Seq or genotyping by sequencing (GBS) protocol to identify SNPs for each species. These methods use restriction enzymes to sample the same set of loci across individuals. DNA samples for each individual are digested using restriction enzymes such as ApeKI, a common-cutting enzyme that works well in plants, and PstI, a rare-cutting enzyme. Following digestion, PCR adapters and unique individual barcodes are ligated to samples, and these gene regions are PCR amplified. Sequencing will be performed on an Illumina or equivalent platform, capable of producing 150-250 bp reads. In order to keep sequencing costs minimal, all samples will be processed and sequenced together in large batches. Resulting reads will be processed and assembled for further genetic analysis.

To examine population genetic structure, genetic differentiation (F_{ST}) and gene flow will be estimated among occurrences and genetic diversity (heterozygosity, number of alleles) estimated within sites. To identify genes under selection we will perform an outlier analysis, which will look for SNPs that are especially differentiated among populations compared to the other SNPs identified. Identified outliers can be taken as evidence of diversifying selection and suggests a possible role in local adaptation. We will also examine genetic differentiation with respect to environmental attributes at sampled locations, such as elevation, soil characteristics, and distance to coast in a mixed model framework.

After the genetic analyses are complete and ploidy levels among the occurrences are determined, a panel of experts in plant ecology, restoration, genetics, and plant management will be convened to make recommendations for each plant species. The panel will identify if there are issues that need to be addressed before management actions are taken such as seed bulking, population enhancement and new population establishment. These issues could include effects on plant fitness arising from ploidy incompatibilities, mixing seed from different occurrences, or establishing occurrences with seed sources that are novel to the environment. If these issues are identified for a rare plant species, then a plan will be developed that identifies the questions to address based on the proposed management action, the type of study that needs to be conducted (e.g., common garden experiment, reciprocal translocation) and the timeline.

Approximate Timeline

Year 1:

April 2016 – August 2016: Collect specimens. Ploidy analysis by flow cytometry for 5 species (Orcutt's Bird's Beak, Saltmarsh Bird's Beak, Willowy Monardella, Otay Tarplant, San Diego Thornmint)

June 2016 – December 2016: Database samples, perform DNA extractions, develop and refine sequencing protocols, and preliminary genomic sequencing analysis for four species (Orcutt's Bird's Beak, Saltmarsh Bird's Beak, Willowy Monardella and Otay Tarplant).

Year 2:

January 2017 – June 1 2017: Ploidy analysis by flow cytometry for 1 species (Encinitas Baccharis) DNA extractions, genomic sequencing and analysis of 2 species (Encinitas Baccharis and San Diego Thornmint).

March 2017 – June 2017: Collect additional specimens.

June 2017 – August 2017: Genomic data analysis and synthesis.

August 2017 – September 2017: Convene expert panel to discuss results and develop recommendations for future research and management actions

September 2017 – December 2017: Complete genomic analysis and prepare report and final panel recommendations.

Deliverables:

- The SDNHM will prepare a summary report with sampling locations, ownership/manager of locations, status of occurrence (present/absent), estimated population size number sampled at each location for each species, habitat information (eg. aspect, soils, elevation), disturbance regime (eg. invasives, fire, off-roading), associate species and vegetation type.

- Voucher sample data will be added to the SD Herbarium database housed at SDNHM. Voucher data will be uploaded to the Consortium of California Herbaria which can be accessed online at <http://ucjeps.berkeley.edu/consortium> Progress Report: a report containing ploidy test results and genomic research progress will be delivered to SANDAG by December 30 2016.
- Rare Plant Genomic Resources Panel: an expert panel or workshop to disseminate and discuss results for rare plant species will be held (targeted for Summer 2017). Implications for management actions and further research needs will be developed.
- Final Report: A report containing final results of all genomic studies, workshop, and resulting recommendations for management of rare plant populations will be detailed in a report delivered to SANDAG by December 30 2017. Report will include database of genomic data to be archived in the SC-MTX Database.

Phase 2 (Common Garden and Reciprocal Transplant Studies): Studies will be designed based on panel recommendations to be carried out beginning in year 3.

Table 1. Information for 6 rare high priority plant species with relevant MSP objectives in 2014-16. Information is provided on the species' distribution, threats, and genetics, with additional questions, estimated number of tissue samples to collect, and other relevant MSP management objectives.

Species	Species distribution and status, life cycle, and relevant genetics information	Additional questions (to those listed above)	# of occ. in MSPA	Target # of samples	Timeline of MSP genetic study objective & other management objectives dependent on the results of the genetics study
San Diego thornmint (<i>Acanthomintha ilicifolia</i>)	SD Co & NW Baja CA. 46 sites with occur. likely to be extant on Conserved Lands in MSPA. Some of these occur. are on private easements. Primary threat is invasive, nonnative plants, particularly annual grasses & forbs (CBI2014). Other large-scale threats include small popn size, altered fire regime, habitat fragmentation, nitrogen deposition, and climate change. At the preserve level vulnerable to trampling, trash dumping, off-highway vehicles, mowing, altered hydrology, and herbivory. Recent genetic study (CNLM 2014) found moderate genetic diversity among 21 sites, pattern of gene flow decreasing as a function of distance & differentiation among popns most related to longitude (elevation). Indications of multiploidy (diploid, tetraploid & hexaploid). CNLM is conducting further common garden expts to determine fitness effects of multiple ploidy levels within popns.		40	800	2016 Genetics study (Adaptive Mgmt Framework for San Diego thornmint, App. 2, p A-33) 2017 Seed bank/bulk – use genetic study results to inform seed collection plan (App 2, p A-34-35) 2016-2017 Prepare Implementation Plan – genetic info. will be used to develop plan to enhance existing occ. & establish new occ 2017-18 Implement Implementation Plan – implement highest priority management actions identified in Implementation Plan 2017-18 Enhance Existing Occurrences – based upon genetic study & other info. identify selected small occur.to enhance that are regionally important for long-term persistence. 2017-18 Establish New Occurrences – based upon genetic study & other info. establish new occur. in suitable habitat that are important for connectivity.
Salt marsh bird's-beak (<i>Chloropyron maritimum</i> ssp <i>maritimum</i> formerly <i>Cordylanthus maritimus</i> ssp <i>maritimus</i>)	SLO south to NW Baja CA (7 specimen records from Baja). 7 isolated occur. at 5 sites in MSPA. Multiple threats, little population info., some may be very small or no longer extant. Annual herb, facultative hemiparasite. Genetics sampled at few populations across range (Tijuana Estuary & Sweetwater Marsh translocated populations in MSPA) in 1995 (Helenurm & Parsons 1997). Transplanted population (now likely extirpated), exhibited low genetic diversity, loss of rare alleles through genetic drift & reduced heterozygosity. Climate change & flooding from sea level rise could pose a challenge to this species & low genetic diversity could compromise response. Polyploidy not recorded, although some perennial species in same subtribe (Castillejinae) exhibit polyploidy (Tank & Olmstead 2009).	1. How does diversity within the MSPA compare to other locations in southern California? 2. Is pollen transfer necessary to increase genetic diversity? (5-Year Review)	7	140	2014-15 Genetics study (Vol 2, P. 2-14 & 2-18) 2015-2016 Seed bank/bulk –genetic study results will be used to guide sampling for seed bank & seed bulking (Vol 2, P. 2-14 & 2-19) 2015 Prepare Implementation Plan - genetic info. will be used to develop plan to enhance existing occ. & establish new occ. (Vol 2, P. 2-19). 2018 Expand Sweetwater & Paradise Marsh occ. as part of Sweetwater Unit CCP (Vol 2, P. 2-20; USFWS 2006) 2019-23 Restore salt marsh habitat & establish new occ. at sites prioritized in Implementation Plan (Vol 2, P. 2-14 & 2-20) (All objectives are 5 Year Review (USFWS 2009) &/or Recovery Plan Recommendations (USFWS 1985)

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Orcutt's bird's-beak (<i>Dicranostegia orcuttiana</i> , formerly <i>Cordylanthus orcuttianus</i>)	SW SD Co & NW Baja CA. (50-60 specimen records from Baja). 5 isolated occ. in MU3 in fragmented habitat. Limited habitat available in seasonal drainages & adjacent upland. All occ. isolated by development & at least 2-4 miles from next closest occ. 1 occ. fairly large, the rest are small. Annual dicot herb, hemiparasite. No info. on genetic diversity or popn structure. Polyploidy not recorded, although some perennial species in same subtribe (Castillejinae) exhibit polyploidy (Tank & Olmstead 2009).	1. Is it hemiparasitic? If so, what is the host plant?	5 (1 large, 4 small)	100	2014-15 Genetics study (Vol 2, P. 2-33 & 2-36) 2015-2016 Seed bank/bulk – occurrence information and genetic study results will be used to guide sampling for seed bank & seed bulking (Vol 2, P. 2-33 & 2-36) 2017 Prepare Implementation Plan – Occurrence and genetic info. will be used to develop plan to enhance existing occ. & establish new occ. (Vol 2, P. 2-37).) 2018 Expand ≥ 3 occur. (Vol 2, P. 2-33 & 2-37). 2019-23 Establish new occ. at site prioritized in Implementation Plan (Vol 2, P. 2-33 & 2-37)
Willow monardella (<i>Monardella viminea</i> , formerly <i>Monardella linoides</i> ssp <i>viminea</i>)	Occurs only in MSPA & Miramar (5 occ. in MUs 4 & 6). Bulk of population is on MCAS Miramar. High degree of threat from erosion, fire, invasive species. For some occ. habitat becoming unsuitable from erosion & scouring. Small number of individuals in occ. Perennial dicot herb. Phylogenetic study split <i>Monardella linoides</i> ssp <i>viminea</i> into two species; <i>Monardella viminea</i> and <i>M. stoneana</i> (Prince 2009). Populations have declined substantially since 2002, potential for genetic bottleneck.		6	280	2014-15 Genetics study (Vol 2, P. 2-67 & 2-72) 2015-2016 Seed bank/bulk occurrence and genetic study results will be used to guide sampling for seed bank & seed bulking (Vol 2, P. 2-67 & 2-72; 5-Year Review Recommendation (USFWS 2012)) 2015 Prepare Implementation Plan – Occurrence and genetic info. will be used to develop plan to enhance existing occ. & establish new occ. (Vol 2, P. 2-67 & 2-74). 2017 Expand existing occur. (Vol 2, P. 2-67-68 & 2-74). 2019-23 Establish 2 new occ. at sites prioritized in Implementation Plan (Vol 2, P. 2-67-68 & 2-74; 5-Year Review Recommendation (USFWS 2012)).

Species	Species distribution and status, life cycle, and relevant genetics information	Additional questions (to those listed above)	# of occ. in MSPA	Target # of samples	Timeline of MSP genetic study objective & other management objectives dependent on the results of the genetics study
Encinitas baccharis (<i>Baccharis vanessae</i>)	SD Co. Entire species distribution in SD Co. 8 occ. at 8 sites in MU's 3, 4 & 6. Dicot dioecious shrub. Very low seed viability, no signs of recruitment reported since 1991 (although not well monitored) & reduced reproductive potential at older age classes (USFWS 2011). Threatened by invasive species, altered fire regime. Very small isolated occurrences (all but 1 are <50 plants) with little connectivity. Inbreeding depression is identified as one potential cause of seed mortality & low reproductive success (USFWS 2011).	1. Are populations inbred?	9	180	2014-15 Genetics study (Vol 1, App. Table 1D; 5-Year Review Recommendation (USFWS 2011)) 2015-16 Conduct study of seed mortality & reproductive success (Vol 1, App. Table 1D; 5-Year Review Recommendation (USFWS 2011)) 2015-2016 Seed bank/bulk – occurrence and genetic study results will be used to guide sampling for seed bank & seed bulking (Vol 1, App. Table 1D) 2017 Prepare Implementation Plan – occurrence and genetic info. will be used to develop plan to enhance existing occ. & establish new occ. (Vol 1, App. Table 1D). 2018 Expand existing occ. (Vol 1, App. Table 1D; 5-Year Review Recommendation (USFWS 2011)). 2019-23 Establish new occ. at sites prioritized in Implementation Plan (Vol 1, App. Table 1D).
Otay tarplant (<i>Deinandra conjugens</i> formerly <i>Hemizonia conjugens</i>)	SW SD Co, Baja CA. (6 records from Baja). Limited distribution; 23 occurrences on Conserved Lands confined to MU's 2 & 3. Threatened by invasive nonnative plants, off-road vehicles, habitat fragmentation & pollinator connectivity. Annual, dicot herb, self-incompatible, requiring pollinators for reproduction. Some closely related species have popns with different chromosome arrangements that are completely intersterile & qualify as distinct biological species even though there are no morphological differences (Baldwin 2006, 2007, B. Baldwin in TNC 2014). Mixing seed between such popns could cause irreversible impacts. Preliminary genetic study indicates <i>D. conjugens</i> has relatively high genetic diversity, although this based on relatively low number of polymorphic loci & small number of popns sampled (Bauder & Truesdale 2000). Study cautions that <i>D. conjugens</i> is susceptible to stochastic changes in allele frequency & strong selection that could decrease genetic diversity & eliminate rare alleles through inbreeding.	1. What is level of variability of “S” locus (prevents self-fertilization) among popns & are there popns that exhibit inbreeding depression & need genetic augmentation? (Recovery Plan Recommendation (USFWS 2004)) 2. Does pollinator connectivity need to be restored for some occ.? (Recovery Plan Recommendation (USFWS 2004))	23	460	2014-15 Genetics study (Vol 2, P. 2-183-184 & 2-189; Recovery Plan Recommendation (USFWS 2004)) 2014-2016 Seed bank/bulk occurrence and genetic study results will be used to guide sampling for seed bank & seed bulking (Vol 2, P. 2-183-184 & 2-190; Recovery Plan Recommendation (USFWS 2004)) 2016 Prepare Implementation Plan – Occurrence and genetic info. will be used to develop plan to improve pollinator connectivity, enhance existing occ. & establish new occ. in conjunction with the South County Grassland Project (Vol 2, P. 2-191). 2014-18 Expand existing occur. as part of South County Grassland Project (Vol 2, P. 2-183-184 & 2-191). 2017-18 Establish new occ. at sites prioritized in Implementation Plan (Vol 2, P. 2-183-184 & 2-191).
		Total Sites and Individuals	90	1960	