The Population and Genetic Status of the Endangered Vernal Pool Annual *Limnanthes floccosa* Howell ssp. *californica* Arroyo: Implications for Species Recovery

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ABSTRACT. Vernal pool ecosystems are in decline throughout California, with only 10% of historic habitat remaining. Loss and fragmentation of vernal pools have decimated populations of many naturally rare and endemic plant species specializing in this unique habitat, with challenges to population and species persistence. As a result, many vernal pool annual plant species are state or federally listed as threatened or endangered, and recovery plans require information on their genetic and ecological status to guide restoration efforts. *Limnanthes floccosa* ssp. *californica* is a vernal pool annual of high conservation concern. Information on its reproductive ecology and conservation genetic status, coupled with long-term annual population and soil seed bank abundance records are needed to develop and implement appropriate restoration and recovery actions. Here I discuss recent studies outlining the low genetic diversity (1.9 [0.06 SE] alleles/locus; H_{obs}= 0.10 ± 0.018, H_{exp}= 0.19 ± 0.015), spatial genetic structure of 20 distinct extant populations (F_{st} = 0.65, P < 0.0001), and high inbreeding rates of remnant populations (F_{it} = 0.94, F_{is} = 0.82). I review available information on this species' ecology and discuss remaining data gaps, presenting a list of recommendations for future recovery strategies. These include collection of seed material from all extant populations for long-term *ex situ* storage and potential future reintroduction; and initiation of focused studies on long-term abundance, reproductive and pollination/pollinator ecology, seed dispersal mechanisms, and seed bank dynamics. Seed movement to restore declining populations should be regulated, and only occur in populations clearly suffering from inbreeding depression, and after considering all available data on microhabitat, pollinator availability and demography. Seeds for restoration should originate from source sites with greater genetic diversity, yet should be confirmed as genetically compatible via *ex situ* tests for at least two generations before introduction.

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INTRODUCTION

A diverse array of vernal pool complexes exists within California grasslands. These seasonally precipitation-filled shallow depressions are underlain by hardpan, clay pan or bedrock (Keeley and Zedler, 1998). In the past century land use change associated with urban development and agriculture caused the loss of 90% of historic vernal pool ecosystems in California resulting in the endangerment of many species associated with vernal pools throughout the state (Holland, 1978; Griggs and Jain, 1983; Keeler-Wolf et al., 1998).

Specialist species have evolved life histories specific to conditions found in vernal pools, coupled with narrow geographic range, such as annual plants in the genus *Limnanthes.* Specialists with highly restricted ranges in areas of California where habitat loss is great, such as *L. floccosa* ssp. *californica* in Butte

County, and *L. vinculans* in Sonoma County, are now facing population declines and associated genetic erosion, leading to loss of adaptive capacity. Ultimately, these species face extinction unless active restoration activities are implemented (Elam, 1998; Foin et al., 1998).

In addition to habitat loss, vernal pool specialists face threats that include 1) competition with non-native plants, especially where competitors cause changes in vernal pool hydroperiod, nitrogen enrichment favoring non-native plants, and habitat degradation such as physical modifications to hydrology, trash deposition, and 2) severe trampling from livestock (Marty, 2005; Barry, 1998; Weiss, 1999). The lack of appropriate conservation management of extant populations can lead to further decline or extinction of conservation target species due to one or more of these threats (Barry, 1998).

Vernal pool annual plants are highly adapted to seasonal variations in environmental conditions and resulting fluctuations in annual seed production. A reservoir of long-lived seed in the soil seed bank allows these annuals to persist through periods of unfavorable conditions, including marginally appropriate conditions when few or no individuals emerge (Nunney, 2002). In favorable years, populations explode and seed set is high. A balance of such 'boom and bust' years assures long-term persistence of such annual species. However, continual disturbance or competition in boom years may result in persistent low seed set, leading to a steady erosion of seed reserves in the soil. A depleted seed bank, when coupled with genetic erosion and reduced adaptive ability, can contribute to local or even species extinction (Griggs and Jain, 1983; Karron, 1987; Lande, 1993; Elam, 1998; Ferguson and Ellstrand, 1999; Reed and Frankham, 2003).

The historic landscape scale distribution of vernal pools has been radically altered throughout California in the last century, resulting in population fragmentation, reduction, and isolation of many vernal pool specialists, particularly in species with limited geographic ranges near urban centers or intensive agriculture (Karron, 1987; Jokerst, 1989; Sloop and Ayres, *in press*). These small and genetically isolated populations are at increased risk of extinction from random catastrophes and demographic and environmental stochasticity (Lande, 1993). Genetic diversity and adaptive capacity of individuals can erode very quickly in isolated and declining populations due to lack of gene flow, genetic drift, and inbreeding depression (Slatkin, 1987; Futuyma, 1986), despite the buffering effects of the soil seed bank (Nunney, 2002).

Vernal pool plant conservation, management, and restoration require assessments of annual range-wide population abundance, site-specific threats, overall genetic viability and isolation, and seed bank size of species at risk (Sloop and Ayres, *in press*). Long-term surveys of population abundance and regular assessments of threats are key indicators of population 'health,' as large populations are less likely to go extinct than small ones in the absence of acute disturbance (Lande, 1993). Fluctuations in annual population size require regular long-term evaluations in order to differentiate human-caused declines from natural fluctuations. Assessment of population genetic diversity and structure elucidate the adaptive capacity and level of isolation of populations within a species' local distribution or its entire range (Karron, 1987; Elam, 1998; Reed and Frankham, 2003).

Assessment of seed bank size is not yet a common practice, yet could predict the persistence of extant populations when coupled with annual abundance, threat, and genetic information (Ferguson and Ellstrand, 1999). There is a growing need for information about conditions necessary to measure and compare soil seed bank diversity in vernal pool endangered annuals. In this context, trials were commenced in 2008, at a *L. vinculans* site, to test a protocol using a serial screening technique wherein soil samples are passed through screens of decreasing pore size assessing the quantity of target species seeds at one site in thirty-six randomly stratified soil cores within an area of dense *L. vinculans* growth at one extant site in 2008 (C. Sloop and H. Brown, unpublished data). The method proved effective and the *L. vinculans* seeds collected were subsequently deposited at Rancho Santa Ana Botanic Garden for long term storage.

The federally and state listed endangered vernal pool annual *Limnanthes floccosa* ssp. *californica* (Butte County meadowfoam) is one example of a vernal pool annual plant in decline. Its extant range is within and near the urban center of Chico, Butte County, and includes many populations vulnerable to off- and on-site impacts in small habitat fragments, on public and private lands, as well as populations within preserves and on mitigation banks that were created or augmented via seed movement from nearby source sites (U.S. Fish and Wildlife Service, 2005). Recent studies have determined population abundance in 1992, 2004 and 2008, species genetic diversity and population structure, and gene flow barriers in parts of the extant subspecies range (Dole and Sun, 1992; Dole unpublished report; Sloop et al., 2011). Sloop et al. (2011) assessed extant genetic diversity and structure by surveying DNA extracted from leaf tissue of 457 individuals from 21 distinct populations using nine polymorphic microsatellite markers. In the absence of available assessments of population seed bank diversity to determine the population genetic composition of more than one year, this information serves to identify populations that are either genetically depauperate, or are evolutionary significant units with unique genetic resources, meriting high conservation or restoration priority. Further, these studies help determine the most appropriate seed source sites for active restoration, and direct *ex situ* seed conservation needs.

SPECIES STATUS

Species Distribution and Conservation Status

Limnanthes floccosa ssp. *californica* is endemic to vernal pools and swales in a narrow 25-mile strip along the eastern flank of the Sacramento Valley from central Butte County to the northern portion of the city of Chico (Arroyo, 1973; Figure 1), with a historic range that has not changed significantly. However, available habitat, occupied areas, and extant populations have declined within the last 30 years (Keeler-Wolf et al., 1998; U.S. Fish and Wildlife Service, 2005). The species was first described by Arroyo (Arroyo, 1973) and extensive research on seed properties of members of the Limnanthaceae (Jain et al., 1978) was followed by field surveys and mapping in the 1980s (Jokerst, 1989). It was state listed as endangered in February 1982, followed by federal listing in 1992 (Federal Register, 1992). The Fish and Wildlife Service designated critical habitat for the species in 2005. Using simple-sequence-repeat (SSR or microsatellite) analysis, Sloop et al. (2011) confirmed regional genetic structuring of three established centers of population distribution ($F_{st} = 0.21$, $P < 0.0001$), previously based on morphology (Jokerst, 1989), and genetic analysis of eight populations using isozyme markers (Dole and Sun, 1992). However, assessing a total of 21 extant populations, Sloop et al. (2011) found 20 to be distinct, including one created population at the northern end of the range (Figure 1, Wurlitzer), and a natural population at Table Mountain at the southeastern distribution boundary, both located beyond the established population centers (Figure 1).

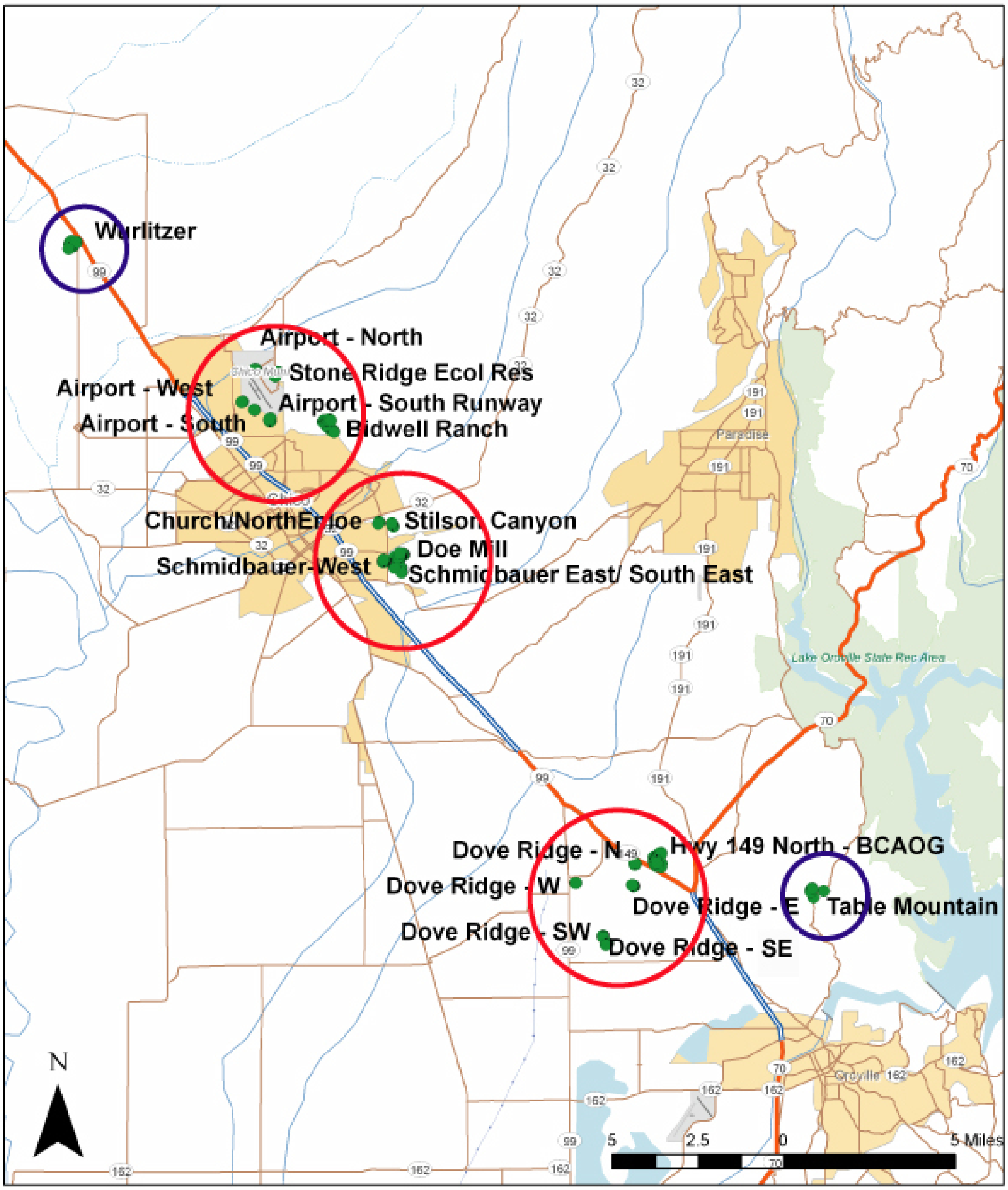

FIGURE 1. 2008 Butte County Meadowfoam (*Limnanthes floccosa* ssp. *californica*) populations sampled in 2008 by Sloop et al. (2011). Large circles indicate three centers of population distribution (North, Northeast, South) described in Dole and Sun (1992); small circles indicate outlying populations to the North (Wurlitzer) and South (Table Mountain).

Reproductive Ecology and Population Abundance

A member of the meadowfoam family (Limnanthaceae), *L. floccosa* ssp. *californica* is a winter annual herb. A mixed breeding system allows the species to self-pollinate in the absence of suitable insect pollinators and effectively outcross in their presence; however, it is considered to be mostly self-pollinating (Arroyo, 1973). Recent genetic analysis found high levels of inbreeding consistent with predominant self-pollination (Sloop et al., 2011). Individual plants have an average of 1.1 to 3.8 flowers, with 2 to 5 nutlets per flower that disperse short distances by water (Hauptli et al., 1978; Rod Macdonald, pers. com.). To date, particular insect pollinators of the species have not been identified (U. S. Fish and Wildlife Service, 2005), but likely include native burrowing bees, honeybees, beetles, flies, true bugs (order Hemiptera), butterflies, and moths (Mason, 1952; Thorp and Leong, 1995). Generally, selfing-ability is a beneficial strategy when pollinators are unavailable over the short term, but it can become disadvantageous over the long term, should insect pollinators disappear permanently. Then, reduced fitness from inbreeding depression can prevent successful reproduction, causing small and declining populations to head toward further decline or extinction (Lynch, 1991; Gilpin and Soulé, 1986). Inbreeding depression may have been purged due to predominant self-pollination over many generations (Hedrick and Kalinowski, 2000; Hedrick, 1994).

Recent and consistent range-wide abundance records of *L. floccosa* ssp. *californica* are sparse (Dole and Sun, 1992; Dole, unpublished report; Sloop et al., 2011) and the comparability of records is complicated by the use of varying population names and incomplete site descriptions. Available estimates indicate steady population numbers at some locations, and severe declines at others (Table 1). Implementation of more rigorous annual monitoring would increase confidence in trends and provide a much-needed baseline for ongoing and future restoration efficacy evaluation. Assessment protocols utilizing citizen science are available for other California vernal pool annual plants, including *L. vinculans* (Sloop and Brown, *in press*) and could easily be adjusted for use with this species.

Seed dormancy in *L. floccosa* ssp. *californica* helps explain population fluctuations of up to two orders of magnitude between years (Dole and Sun, 1992; Jokerst, 1989; Ritland and Jain, 1981), indicating that much of the species' genetic variation and potential adaptive capacity may be stored in the seed bank.

Genetic Diversity, Structure and Gene Flow

Regional scale genetic analyses of 21 populations utilizing microsatellite markers (F_{st} = 0.21, $P < 0.0001$) supported the existence of three *L. floccosa* ssp. *californica* population distribution centers (Sloop et al., 2011), as previously reported by Dole and Sun (1992) studying eight populations with isozyme markers. However, population scale microsatellite analysis showed that 20 out of 21 tested extant populations were genetically distinct (F_{st} = 0.65, $P < 0.0001$, Table 2), indicating strong genetic structure among all populations (Sloop et al., 2011). This also indicates that even within distinct centers of distribution, gene flow is limited among populations. Using Bayesian ordination Sloop et al. (2011) found only two populations located within the Dove Ridge Conservation Bank (southwest and southeast) to be genetically similar, yet they significantly differed from the remaining populations at the site (east, west, north), suggesting gene flow occurs at the range of 0.25 kilometers, but is reduced over longer distances. Population isolation was significantly influenced by geographic distance (R^2 = 0.22, $p < 0.01$, Sloop et al., 2011), and there were six significant gene flow barriers that divided populations within and between centers of distribution and from the two outlying populations (Table Mountain and Wurlitzer; Sloop et al., 2011).

The low gene flow throughout the range and between neighboring populations, coupled with inbreeding, could explain the high level

TABLE 1. Population estimates of extant *Limnanthes floccosa* ssp. *californica* populations in 1992, 2004, and 2008. Superscript letters indicate site names different from those listed, and infer that listed estimates may not reflect counts at the same population. Populations were separated by at least 0.25 km.

	Population Size Estimates (Observation Year)		
Location of Populations	Dole and Sun (1992)	Dole (2004)	Sloop (2008)
Bidwell Ranch	na	~553[a]	~5,000
Airport - North	~7,819	96	105
Airport - South	~1,520	103	36
Airport - South Runway	na	na	81
Airport - West	~1,000	18	47
Church	na	na	~1,660
Doe Mill Reserve	8,713	552	8,177[b]
Dove Ridge - East	na	320	~600
Dove Ridge - North	na	480	~365
Dove Ridge - SE	na	760	~1,000
Dove Ridge - SW	na	780	~2,000
Dove Ridge - W	na	260	~159
Hwy 149 North	na	na	~802
North Enloe	~45,689	624	~1,065
Schmidbauer - East	na	na	~1,365
Schmidbauer - South East	na	na	~200
Schmidbauer - West	~354	~10	~452
Stilson Canyon Road	~22,980	na	~500
Stone Ridge Ecological Reserve	~220[c]	~1,007[d]	~1,000
Table Mountain	na	na	~210
Wurlitzer	na	na	6,078[b]

[a] Site name in unpublished Dole report: West Rancho Arroyo.
[b] Data provided by Rod Macdonald.
[c] Site name in Dole and Sun (1992): Airport Northeast.
[d] Site name in unpublished Dole report: Cohasset SW and NE.

of genetic structure found by Sloop et al. (2011). The low allelic diversity of 1.9 ± 0.06 alleles/locus in *L. floccosa* ssp. *californica* (Sloop et al., 2011), especially when compared to 7.4 ± 3.8 alleles/locus in *L. vinculans* (Ayres and Sloop, 2008), and the elevated inbreeding coefficient (F_{is} = 0.82, P < 0.00001; F_{it} = 0.94, P < 0.00001; Sloop et al., 2011) support the reported prevalence of self-fertilization or predominant inbreeding in this species. Evidence for populations having undergone a genetic bottleneck (a temporary decline in population size causing the loss of genetic variation) was tested using Wilcoxon sign rank tests in BOTTLENECK v. 1.2.02 (Piry et al., 1999), and existed only for two populations under the step-wise-mutation model: Doe Mill (P = 0.015), and Schmidbauer East (P = 0.055; Sloop et al., 2011). Unique individual genotypes varied by population at levels from 10% to 84% (Sloop et al., 2011), and population specific inbreeding coefficients indicated that individuals at some sites are more inbred than at others (Table 3). In some populations individuals varied only at one or two genetic loci (Airport South, Airport West, Dove Ridge Conservation Bank North), while in others individuals varied at seven or eight out of nine loci (North Enloe, Schmidbauer East, Highway 149, and Table Mountain), with individuals in the remaining 14 populations varying at three to six out of nine loci (Sloop et al., 2011).

The documented levels of population genetic variation and structure in *L. floccosa* ssp. *californica* are likely the result of: 1) high rates of inbreeding, 2) significantly reduced natural dispersal of pollen and seeds across an increasingly degraded and fragmented landscape, reducing diversity and increasing structure, 3) remnants of temporal gene flow from reserves in the soil seed bank, and 4) perhaps even increased gene flow due to human restoration activities (e.g., similarity between two mitigation sites at Wurlitzer and Hwy 149 north; Sloop et al., 2011), with the potential to increase diversity and lower genetic structure.

Recommendations for Species Recovery

Recent studies on population abundance and genetic status of *L. floccosa* ssp. *californica* assessed most known extant populations throughout the range and showed that five populations were at very low population levels in 2008 (n < 200, Table 1). For the remaining populations, levels in 2008 consisted of n = ~200 to n =~8,000 individuals, in some cases holding steady from 1992 counts, yet for most populations only 2008 abundance data exist (Table 1). It is crucial to consider all remnant populations as high priorities for conservation and to target populations at low abundance levels for active restoration, since many of the remaining Butte County vernal pool landscapes face threats from urbanization. Of the 21 tested populations 95% were genetically distinct (Sloop et al., 2011). Populations for potential active restoration via carefully planned and highly regulated seed introduction efforts include all four Chico Airport and Dove Ridge Conservation Bank North and West populations (Sloop et al., 2011).

Because all remnant populations must remain high conservation priorities, seeds from all extant populations should be collected throughout each flowering season, over several years for long-term *ex situ* storage and potential future reintroduction. Introducing new genetic information via seeds into an inbred and declining population, can increase population genetic diversity and, in theory, adaptive capacity. However, outbreeding depression, or the incompatibility of divergent lineages, can cause the disassociation of important genetic complexes, and can result in the collapse of the next generation (Lynch, 1991; Fenster and Galloway, 2000; Waser et al., 2000; Edmands, 2007). Thus, the compatibility of lineages should be tested *ex situ* on a small

TABLE 2. Pairwise Population Fst Values. Fst Values are shown below diagonal. Probability values based on 1000 permutations are shown above diagonal; for methods see "Analysis of Molecular Variance" in Sloop et al. (2011). **APN** – Airport N, **APS** – Airport S, **APSR** – Airport S Runway, **APW** – Airport W, **BR** – Bidwell Ranch, **CH** - Church, **DM** – Doe Mill, **HWY** – Hwy 149 N, **DRCBe** – Dove Ridge E, **DRCBn** – Dove Ridge N, **DRCBse** – Dove Ridge SE, **DRCBsw** – Dove Ridge SW, **DRCBw** – Dove Ridge W, **NE** – North Enloe, **SCHe** – Schmidbauer E, **SCHse** – Schmidbauer SE, **SCHw** – Schmidbauer W, **SR** – Stone Ridge, **STN** – Stilson Canyon, **TM** – Table Mountain, **WU** – Wurlitzer.

	APN	APS	APSR	APW	BR	CH	DM	HWY	DRCBe	DRCBn	DRCBse	DRCBsw	DRCBw	NE	SCHe	SCHse	SCHw	SR	STN	TM	WU
APN	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
APS	0.541	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
APSR	0.428	0.451	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
APW	0.525	0.729	0.693	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
BR	0.364	0.488	0.433	0.360	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CH	0.424	0.352	0.309	0.465	0.274	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DM	0.295	0.475	0.409	0.287	0.114	0.248	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
HWY	0.397	0.468	0.415	0.452	0.424	0.333	0.378	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DRCBe	0.419	0.410	0.295	0.556	0.475	0.252	0.414	0.393	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DRCBn	0.658	0.752	0.730	0.823	0.719	0.604	0.674	0.447	0.598	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DRCBse	0.509	0.547	0.544	0.593	0.482	0.361	0.428	0.346	0.450	0.526	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DRCBsw	0.529	0.572	0.523	0.556	0.558	0.370	0.489	0.335	0.343	0.571	0.366	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DRCBw	0.602	0.715	0.676	0.807	0.683	0.518	0.618	0.323	0.453	0.664	0.523	0.461	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
NE	0.340	0.368	0.198	0.351	0.215	0.134	0.232	0.328	0.217	0.602	0.429	0.351	0.507	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SCHe	0.261	0.538	0.477	0.327	0.267	0.392	0.185	0.357	0.484	0.694	0.502	0.520	0.651	0.312	**0.000**	0.001	0.001	0.001	0.001	0.001	0.001
SCHse	0.431	0.514	0.505	0.191	0.198	0.311	0.120	0.418	0.470	0.757	0.507	0.513	0.716	0.283	0.268	**0.000**	0.001	0.001	0.001	0.001	0.001
SCHw	0.441	0.527	0.522	0.382	0.124	0.274	0.127	0.446	0.451	0.740	0.451	0.556	0.703	0.296	0.306	0.163	**0.000**	0.001	0.001	0.001	0.001
SR	0.425	0.521	0.427	0.465	0.384	0.411	0.310	0.418	0.479	0.688	0.549	0.557	0.670	0.359	0.313	0.356	0.402	**0.000**	0.001	0.001	0.001
STN	0.560	0.516	0.458	0.496	0.307	0.306	0.300	0.428	0.487	0.787	0.550	0.543	0.757	0.204	0.419	0.292	0.409	0.451	**0.000**	0.001	0.001
TM	0.476	0.527	0.555	0.487	0.379	0.355	0.291	0.481	0.492	0.698	0.478	0.544	0.634	0.410	0.424	0.370	0.328	0.463	0.492	**0.000**	0.001
WU	0.457	0.567	0.439	0.461	0.446	0.371	0.420	0.219	0.430	0.662	0.490	0.362	0.456	0.252	0.419	0.439	0.495	0.479	0.431	0.539	**0.000**

scale, before field introductions are made.

Rules for population crosses to increase genetic diversity, or augment one declining population with seeds from another should include:

- Seed movement to restore declining populations should only occur in populations clearly suffering from inbreeding depression, and only after considering all available data on microhabitat, pollinator availability and demography (Edmands, 2007).
- Seeds used in restoration should originate from source sites with greater genetic diversity than the target site, yet that have been shown as genetically compatible (i.e. located in the same center of distribution/gene flow area), and where the effects of crosses have been tested *ex situ* for at least two generations, whenever possible (Edmands, 2007).

The identification and recovery of pollinators to allow sexual recombination may play a decisive role in the long-term persistence of *L. floccosa* ssp. *californica*. Future work should examine seed set and plant survivorship under self- and out-cross pollination to determine if the species is undergoing inbreeding depression, and whether manual pollen cross-fertilizations can be utilized to introduce gene flow in inbred populations. Population-specific inbreeding coefficients indicate the least inbred (i.e., Table Mountain, Table 3) and the most inbred (i.e., Airport South, Table 3). Field studies to compare and contrast pollinator activity and seed bank size with level of genetic diversity can be implemented to show whether populations with a smaller inbreeding coefficient have greater pollinator service, and whether their seed bank is larger and more genetically diverse. This will also help to establish if insect pollinators exist for *L. floccosa* ssp. *californica*.

It remains to be determined whether and at what level seed (or pollen) movement by human activities would be beneficial for the recovery of all extant populations, especially those of low population size. At Wurlitzer, a substantial number of plants and seeds were moved more than a decade ago from a now developed site adjacent to Doe Mill to inoculate this mitigation site (Rod Macdonald, pers. com.). Wurlitzer *L. floccosa* ssp. *californica* are now genetically divergent from several populations within its historic range and more

TABLE 3. Population specific inbreeding coefficients (F_{is}). Probability estimates based on 1023 permutations; for methods see 'Analysis of Molecular Variance' in Sloop et al. (2011). **APN** – Airport N, **APS** – Airport S, **APSR** – Airport S Runway, **APW** – Airport W, **BR** – Bidwell Ranch, **CH** – Church, **DM** – Doe Mill, **DRCBe** – Dove Ridge E, **DRCBn** – Dove Ridge N, **DRCBse** – Dove Ridge SE, **DRCBsw** – Dove Ridge SW, **DRCBw** – Dove Ridge W, **HWY** – Hwy 149 N, **NE** – North Enloe, **SCHe** – Schmidbauer E, **SCHse** – Schmidbauer SE, **SCHw** – Schmidbauer W, **SR** – Stone Ridge, **STN** – Stilson Canyon, **TM** – Table Mountain, **WU** – Wurlitzer.

Site	Fis	P
DRCBn	1.00000	0.00000
APS	1.00000	0.00000
APW	1.00000	0.00000
DRCBe	0.99999	0.00000
WU	0.99999	0.00000
APN	0.99999	0.00293
BR	0.99999	0.00000
APSR	0.99999	0.01955
DRCBse	0.97410	0.00000
SCHw	0.95825	0.00000
DM	0.95204	0.00000
HWY	0.88176	0.00000
SCHse	0.87850	0.00000
NE	0.86700	0.00000
SCHe	0.85827	0.00000
STN	0.83908	0.00000
CH	0.82995	0.00000
DRCBw	0.78689	0.00587
DRCBsw	0.68116	0.00196
SR	0.63540	0.00000
TM	0.31089	0.00098

closely resemble another population (Hwy 149 North) on a mitigation site at the opposite end of the range that perhaps received similar seed inoculum (Sloop et al., 2011). The feasibility and effectiveness of human-induced gene flow into declining populations is controversial and should be further evaluated. Small-scale greenhouse trials may allow the evaluation of cross-pollination success and determination of outbreeding depression between individuals grown from seeds from a declining site and those originating from potential source sites. After lineages are considered compatible, cross-pollinated seeds from the resulting lineages of such trials may then be returned to the restoration site, noting relative survival *in situ*.

Seed bank sizes should be assessed at all extant sites to determine a baseline of site-specific conservation and restoration potential. There seems to be effective gene flow between some populations (Sloop et al., 2011), and there is sufficient evidence that all of the extant sites with their varying microhabitats should be conserved to maintain the highest possible level of genetic diversity of this naturally rare and endemic species.

A database of the available genetic, and long-term demographic and ecological information (including potential threats) of each extant population should be developed and maintained into the future to inform adequate long-term population management range-wide. Volunteer based, guided citizen science surveys of endangered plants are a potential way to implement long-term surveys, as currently conducted in the Santa Rosa Plain vernal pool complex (Sloop and Brown, *in press*). Long-term annual status determinations, including abundance, seed set and seed bank status data, coupled with site-specific information on threats for use in population viability analyses, are critically important to inform the appropriate conservation, restoration and management actions aimed at the recovery of populations and the entire species.

A science advisory panel should be created and consulted to effectively direct the important long-term restoration and conservation process of *L. floccosa* ssp. *californica*. In order to attain a better understanding of gene flow among populations, focused studies on the reproductive and pollination/pollinator ecology, seed dispersal mechanisms, and seed bank dynamics should be undertaken as quickly as possible.

List of Recommendations

1) Include all remnant populations as high priority conservation targets.

- Conserve all microhabitats to maintain the highest possible level of genetic diversity.
- Consider a subset of remnant populations for active restoration.

2) Collect seed material from all extant populations for long-term *ex situ* storage and potential future reintroduction. (To maximize genetic diversity, collections should occur throughout each flowering season—early, mid and late season flowers—and over several years.)

3) Initiate focused studies on long-term abundance, the reproductive and pollination/pollinator ecology, seed dispersal mechanisms, and seed bank dynamics as quickly as possible.

- Implement long-term status surveys utilizing citizen science volunteers.
- Assess seed bank sizes at all extant sites as a baseline of site-specific conservation and restoration potential.
- Determine if active insect pollinators still exist for this species, and determine pollinator activity, contrasting least and most inbred populations.

- Evaluate potential seed dispersal mechanisms.

4) Evaluate the feasibility and effectiveness of human-induced gene flow into declining populations. Examine seed set and plant survivorship in self- and out-cross pollination trials to determine if:

- the target population is undergoing inbreeding depression, and/or
- manual cross-fertilizations can be utilized to introduce gene flow in inbred populations.

5) Initiate active restoration via carefully planned and highly regulated seed introduction efforts (i.e., in Chico Airport or in Dove Ridge Conservation Bank North and West populations).

- Test the compatibility of individuals grown from seeds from genetically similar populations in greenhouse trials for at least two generations.
- Slowly increase seed crosses from similar source populations over time to limit outbreeding depression.
- Initiate small-scale greenhouse trials to evaluate cross-pollination success between individuals grown from seeds from declining and from potential source sites.
- Slowly return cross-pollinated seeds from such trials to target restoration site, evaluating relative survival *in situ*.

6) Request long-term, range-wide population management.

- Establish and maintain a database of available genetic, and long-term demographic and ecological information (including potential threats) of each extant population.
- Create a science advisory panel to effectively direct long-term restoration and conservation.

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