Application of Molecular Techniques to Examine the Genetic Structure of Populations of Butte County Meadowfoam (*Limnanthes floccosa* ssp. *californica*)

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Abstract

The endangered annual endemic Butte County meadowfoam (BCM, *Limnanthes floccosa* ssp. *californica*) is restricted to vernal pools along the eastern flank of the Sacramento Valley from central Butte County to the northern portion of the City of Chico. Within the last 30 years known BCM populations were subject to urban development, airport maintenance activities, conversion of agricultural lands to other uses, and road widening or realignment. The relatively small number of remaining extant sites, and results from this and a previous isozyme study on a subset of sites suggest that the loss of any populations may represent a significant deficit of the total amount of genetic variability for the species, making BCM extremely vulnerable to chance catastrophes. BCM recovery will require conservation and restoration of existing populations and protection of their habitat. Knowledge of the extant genetic composition is essential to more appropriately design reintroduction efforts during establishment of new and restoration of declining populations, and to identify populations with particularly unique genetic resources. Analysis of the genetic diversity (within population allelic variation) and regional genetic structure (among population allelic variation) of BCM populations will inform our conservation decisions with respect to possible translocation of individuals (i.e., seeds or plants) from one area to another to recover critically declining populations, and to guide the design of possible seed collection scenarios for long-term *ex situ* seed storage. To examine fine scale and range-wide genetic structure we genetically surveyed 457 individuals from 21 known geographically separate (> 0.25 kilometers apart) extant BCM occurrences using 9 polymorphic microsatellite markers adapted from a suite of markers developed for *Limnanthes alba*. Despite utilizing a highly polymorphic marker system our data confirmed earlier accounts of low within population genetic diversity: average allelic diversity = 1.9 (0.06 SE) alleles per locus; average H obs = 0.10 ± 0.018, average H exp = 0.19 ± 0.015, mean Shannon's information index 0.317 ± 0.025, mean fixation index 0.556 ± 0.044. The number of polymorphic loci ranged between 11% and 89%, with an average of 55% among populations. Bayesian ordination determined 20 distinct population clusters, and we confirmed high genetic structure among these populations (Fst = 0.65, P < 0.000). We identified notable barriers to gene flow across

genetically distinct BCM populations and confirmed evidence for regional structuring between three previously defined centers of population density and two outlying populations ($F_{st} = 0.21$, $P < 0.000$). Population size estimates for all collection sites ranged between ~50 and >5000 extant plants per site. All four Chico airport occurrences showed extremely low population numbers and had notably declined from population levels reported in 1992. We recommend close examination of the microhabitats of these and other declining sites and potential genetically similar seed source sites to determine the potential for human assisted gene flow via seed movement.

INTRODUCTION

In most species, the loss and degradation of their habitats is the main cause of population decline (Foin *et al* 1998). Throughout California, 90% of vernal pool habitat has been lost due to land use changes in the past 50 to 100 years (Holland 1976). Extensive agricultural and urban development of vernal pool habitat throughout California has caused the endangerment of many of the naturally rare and endemic vernal pool plants. Many of these plants now occur in remnants of the formerly vast expanses of the vernal pool landscape and may have suddenly or gradually lost their connectivity to remaining surrounding occurrences as pollinators declined and barriers to gene flow increased.

Over time, adaptation, genetic drift, gene flow and natural selection cause the differential distribution of genetic variation within or among populations of the same species, which is called their population genetic structure (Slatkin 1987, Futuyama 1986). The dispersal of pollen and seed are the main determinants of gene flow. When gene flow is reduced during isolation, populations, defined as groups of individuals within a defined geographic area (here we define distinct populations as pools containing BCM that occur at a distance of greater than 0.25 km distance from each other), can diverge genetically due to natural selection, or the chance effects of genetic drift, and suffer from the negative effects of inbreeding depression. Conversely, the genetic structure among populations is reduced when gene flow is common.

The degree of genetic isolation between populations and the potential for small-scale changes in allele frequencies in a population over a few generations (= their micro-evolutionary potential) can be assessed by analyzing differences in genetic diversity within and genetic structure between populations of plants using selectively neutral markers, and taking into account their breeding system and observations of potential causes of and barriers to gene flow. Information on the genetic diversity and structure of populations can be used to inform conservation efforts to maintain or simulate ecological phenomena that have played a role in the micro-evolutionary development of the species. It can further inform our conservation decisions regarding the possible translocation of individuals (i.e., seeds or plants) from one area to another, and guide the design of possible seed collection scenarios for *ex situ* seed storage.

Limnanthes floccosa ssp. *californica* (Butte County meadowfoam (BCM)) is an herbaceous annual plant that inhabits vernal pool and swale habitats near the town of Chico in California's central valley. Along with three other *Limnanthes* species BCM is Federally and State listed as endangered. Extant populations of BCM plants are geographically isolated, with many occurrences persisting in small habitat fragments that are vulnerable to off-site impacts, occurring on privately owned sites, on recently established preserves, or on mitigation banks in remnant natural pools or where they have been seeded into newly created swales. Most remaining natural populations may now be more isolated from each other, potentially beyond the reach of historic pollinators or seed dispersal mechanisms.

In a previous population genetic survey of BCM Dole and Sun (1992) used 28 isozymes to infer genetic structure of all ten known or discovered populations/sub-populations in 1988. They found extremely low numbers of polymorphic loci per population (ranging between 0% and 3.6%), and reported extremely large genetic structure between populations, with 96% of genetic variation distributed among populations (Gst = 0.96; Dole and Sun 1992). At the time population numbers were restricted in area yet relatively large (ranging between 220 and 45,689), however, despite 500 sampled individuals they were able to distinguish only 5 multilocus genotypes (Dole and Sun 1992), rendering this measure of genetic variation as largely inflated. Isozyme analysis further showed extremely low levels of within population gene diversity (Hs = 0.003 ± 0.003 (SE), Dole and Sun 1992). These findings indicated isolated clusters of genetically dissimilar populations and led to the development of a species conservation plan (Jokerst 1989).

In order to more effectively uncover potential genetic distinctions between individuals, and within and among populations a more variable or polymorphic marker system is needed in order to update the available genetic data on BCM in the face of increasing development pressures throughout its range. The U. S. Fish and Wildlife Service (USFWS) and the Butte Environmental Council therefore commissioned this additional study on an expanded number of extant populations, using a highly polymorphic SSR (single sequence repeat or microsatellite) genetic marker system. SSRs are co-dominant and highly variable or polymorphic repeat regions of two or more

nucleotides of nuclear DNA. For this reason and the high level of repeatability SSR markers currently represent the marker of choice for population genetic studies (Holton 2001, Hacker 2001, Goldstein & Schloetterer 1999). A suite of polymorphic SSRs were recently developed for *Limnanthes alba* (Kishore et al 2004), and a subset of these markers were recently adapted to successfully assess population genetic structure in *Limnanthes vinculans* (Ayres & Sloop 2008). Here, we successfully adapted 9 SSR markers from the same suite developed by Kishore et al. (2004), initially selecting from 42 loci shown as highly polymorphic and heterozygous in *L. alba,* to examine fine scale and range wide genetic structure in *Limnanthes floccosa* ssp. *californica.*

Using this SSR marker technique will allow us to accurately inform recovery and long-term conservation efforts as to the within and among population genetic distinctiveness of the majority of the extant populations (Appendix A & B). This will be invaluable information for directing seed banking and *ex situ* conservation efforts, and to infer possible natural explanations for genetic subdivisions to aid in management decisions, such as the movement of seeds from remnant natural sites to inoculate declining populations, and/or created mitigation pools.

Our goals here were to investigate the genetic diversity within extant populations of BCM and determine the genetic structure across its extant range using a highly polymorphic SSR marker system with the promise to readily uncover existing genetic variation with a large number of unique multilocus genotypes. We hypothesize that highly polymorphic SSR markers will uncover higher levels of genetic diversity within extant populations as compared to those previously estimated with isozymes (Dole and Sun 1992). This will allow more accurate estimation of population differentiation or the degree of genetic structure between populations, as individual genotypes can be distinguished more readily and their relative allelic differences effectively compared. We predict that because of the suggested high levels of self-pollination in BCM (Arroyo 1975, Dole and Sun 1992), and the notable level of decline and isolation of some of the extant populations the within population genetic diversity will be similarly low, and there will be substantial genetic structure. Specific aims include measuring levels of genetic variability within populations, identifying levels of genetic differentiation among

populations, and providing guidelines for the conservation, management, and restoration of the species.

METHODS

Study Species

Butte County meadowfoam (BCM, *Limnanthes floccosa* ssp. *californica*) is a winter annual herb of the false meadowfoam family (Limnanthaceae). It is a densely hairy plant with stems ranging from 1 to 10 inches in length, that generally lie flat on the ground with the tips curved upward. Stems have few leaves in the flowering stage, and fragrant flowers are white with dark yellow veins at the base of each of the five petals. BCM typically begins flowering in February, reaching its flowering peak in March, and if conditions are suitable may continue into April or May (Jenny Marr, DFG, pers comm.). Plants die back by early May after nutlets are produced in March and April (Jokerst 1989; Dole and Sun 1992). Individual plants contain between 1.1 to 3.8 flowers on average, depending on environmental conditions in a given year (Rod McDonald, pers. com.). Each flower or capsule contains between 2-5 nutlets (Rod McDonald, pers. com.). Nutlet dispersal occurs by water, mostly only short distances (Hauptli et al. 1978). Population fluctuations of up to two orders of magnitude between years have been observed at some of its reported locations and could be explained by seed dormancy (Dole and Sun 1992; Jokerst 1989; Ritland and Jain 1984).

Butte County meadowfoam is both adapted to cross-pollination by insects and self-pollination (Arroyo 1975). Because the sepals are partially fused by cottony hair that prevents the flowers from fully opening, it is thought that the plant is mostly self-pollinating (Hickman 1993), and self-pollination may take over to ensure seed set if insect pollination is unsuccessful. To date, the particular pollinators of Butte County meadowfoam have not been identified, but the species is likely pollinated by native burrowing bees, honeybees, beetles, flies, true bugs (order Hemiptera), butterflies, and moths as other meadowfoam species (Mason 1952; Thorp and Leong 1998).

One day before the stigma is receptive the stamens begin shedding pollen, preventing flowers from self-pollination during this period. Thereafter, however, if pollen

remains in the anthers when the stigma matures, gravity can carry it to the stigma, situated below the anthers (Arroyo 1975). Depending on the size of insect populations the rate of self-pollination may vary among years or among sites. Cross-pollination by insects would allow opportunities for genetic recombination, unlike self-pollination, and allow for an increased influx of new alleles into the population to increase its adaptive potential to chance catastrophes (Frankham et al 2002). Further genetic analysis will confirm which type of breeding system is active in extant BCM populations. The earlier isozyme analysis indicated a high degree of inbreeding (Dole and Sun 1992), pointing to selfing as the main mechanism for seed set. Although most populations of Butte County meadowfoam have bisexual flowers, it has been reported that the population at the type locality (at Shippee Road, Appendix B) contained a small percentage of male-sterile plants (Dole and Sun 1992) which could only be successfully pollinated via out-crossing by insects (USFWS 2005). We were not able to locate this population in 2008 and fear that it may be extinct as Clif Sellers reported it as degraded due to agricultural and off road vehicle activities in January 2008 (Appendix B).

Butte county meadowfoam is found in three types of seasonal wetlands: 1) at the edges of vernal pools or swales, 2) occasionally around the edges of isolated vernal pools, and 3) along ephemeral streams (Arroyo 1975; Dole 1988; Jokerst 1989). BCM is generally found on terrain that is level or gently sloping. It occurs on poorly drained soils with shallow soil layers impermeable to water infiltration and also thrives in waterlogged soils and tolerates periodic submergence (Dole and Sun 1992; Jokerst 1989).

BCM is endemic to Butte County, and is restricted to 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). The historic range has not changed significantly, but the amount of available habitat, the area occupied, and extant populations have been negatively impacted and have declined within the last 30 years (USWFS 2006b). BCM has never been extensive in range, but its limited occurrences have been reduced and fragmented mainly by development in the Chico area in the last decades (Keeler-Wolf et al. 1998, Appendix B). All remaining known populations of BCM are still subject to urban development, airport maintenance activities, conversion of

agricultural lands to other uses, and/or road widening or realignment (Finn 2000; USWFS 1992; USFWS 2005).

BCM still occurs in several natural centers of concentration within its range (Appendix A & B). Six extant occurrences are in the southern Shippee Road area between Chico and Oroville near the intersection of Highways 99 and 149, including five occurrences within the Dove Ridge Conservation Bank south of Highway 149, and one occurrence north of Hwy 149 at a Butte county Association of Governments (BCAOG) mitigation site. Six extant occurrences are near Chico, and five are near the Chico Municipal Airport to the north. A single (but relatively widely distributed) occurrence is on Bidwell Ranch, and a new location was found in 2005 on California Department of Fish and Game managed property on North Table Mountain east of the intersection of Highways 149 and 70. An experimental population of Butte County meadowfoam (originating from a now developed site north of the Doe Mill Preserve by inoculation with 70,000 seeds from ~2,000 plants & soil scraped from a developed site north of Doe Mill and Schmidbauer east (Rod McDonald, pers. com.)) was successfully introduced on the Tuscan Preserve (=Wurlitzer site) in northwestern Butte County, just outside of the historical range of the taxon (Kelley et al. 1994, USFWS 2005).

Previous studies have suggested that several races (a northern and southern race) of Butte County meadowfoam exist based on morphology (Jokerst 1989) and using an isozyme marker system (Dole and Sun 1992). Using this marker system Dole and Sun (1992) further identified genetically distinct northern, northeastern and southern races of BCM. Dole and Sun (1992) found very little diversity within populations, especially populations with small numbers of individuals, potentially the result of past population bottlenecks and subsequent inbreeding, yet also in part due to their low resolution of individual multilocus genotypes. When populations are reduced to only a few individuals at a certain time in their history, deleterious alleles are purged and only few individuals pass on their genes to subsequent generations causing a significant reduction in allelic diversity that can have a strong legacy effect if gene flow remains low, such as when inbreeding is frequent. The longer populations remain isolated and the within population allelic diversity remains low, the higher the chance for genetic structure between populations.

Sampling Design

All BCM DNA samples were obtained between March 24, 2008 and April 2, 2008 by collecting leaf samples at a majority of the known extant sites (Figure 1, Appendix A). Within this period all BCM populations were at their peak flowering stage, making species identification easy. Occurrences were distinguished as individual populations by a geographic distance of at least 0.25 kilometers. Table 1 gives a summary of the 2008 sample collections, and Figure 1 shows the map locations of all sampled populations.

Each extant population was surveyed to estimate population size by counting BCM plants within 20 x 50 cm quadrats at regular intervals along a transect throughout the geographic extent of the occurrence. In most cases I was guided by local experts to known BCM locations. Except in a few instances, my population counts were constrained to these known occurrence areas, and in most cases an additional thorough search of the entire property was impossible due to time constraints. An exception to this occurred at the four Chico airport sites, where population numbers were very low, and in order to sample these sites genetically we needed to assess the feasibility of collecting. In most cases sample collections were limited to leaf or stem samples, unless the small size of plants required taking an entire individual in order to have enough sample tissue for successful DNA extraction. At the Doe Mill and Wurlitzer sites Rod Macdonald has surveyed the entire population size for several years. His plant counts were used to determine population size at the Wurlitzer preserve, and we estimated population size at the Doe Mill site by dividing the capsule count determined by Rod Macdonald by 2.45, the average number of capsules (= flowers) per plant.

Plants at each site typically occurred in discrete associations within either a single or within multiple adjacent vernal pools or swales. At each location, according to plant abundance and within site distribution, we collected between 10 and 46 plant tissue samples (~ 30 on average per pool) for genetic analysis (Table 1). A plant tissue sample consisted of one to two leaves or stems, which were placed into individually labeled zip-

lock bags. Plants were collected haphazardly from throughout each site by walking linear or circular transects throughout the target plant's area of distribution and collecting a plant sample at equidistant intervals to cover the complete distribution area (this was variable in each case due to changing distribution densities from site to site). Pool circumference, transects, and sample points were noted using GPS (geographic positioning system). Due to the number of samples, and the fragile nature of the tender stems, plant samples were processed and frozen as quickly as possible and then placed into a -80^{o} C freezer at the Genomics Laboratory at Sonoma State University, Rohnert Park, CA until DNA extraction.

SSR Primer Development

To develop a suite of informative SSR markers for this study we screened a total of 42 highly polymorphic primers with high rates of heterozygosity in test populations of *L. alba* characterized by Kishore et al. (2004). We were able to effectively adapt nine polymorphic markers from this suite of published markers that were suitable for genotyping with Butte County meadowfoam: LS02, LS43, LS122, LS164, LS166, LS179, LS184, LS321, LS527. These markers yielded between 3 and 8 alleles per locus in BCM, being on average half the allele numbers found in 14 accessions of *L. alba* for the same loci (Table 4a). The remaining tested markers were either monomorphic in *L. floccosa* ssp. *californica* (showed no allelic variation among individuals), did not show consistent repeatable bands, or needed a significant amount of further optimization.

SSR Genotyping

DNA Extraction Protocols and Polymerase Chain Reaction (PCR) Conditions

PCR reactions were performed according to the methods in Kishore et al (2004), and using fluorescently labeled forward primers. PCR products were then sized using an ABI 3730 96-capillary DNA analyzer and ABI GeneMapper 3.0 software (Applied Biosystems, Cupertino, CA). Using nine polymorphic SSR markers we successfully genotyped 457 individuals ranging between 9 and 37 individuals each from 21 occurrences, and found a total of 309 and 247 unique mutlilocus genotypes (Table 2).

Only individuals with high quality genotyping data at a minimum of seven or more marker loci were included in the final analysis.

Data Analysis.

Hardy-Weinberg Equilibrium, Linkage Disequilibrium, Null alleles

All samples were tested for deviations from Hardy-Weinberg equilibrium using ARLEQUIN version 3.01 (Excoffier et al 2005) using Fisher's exact test (100,000 Markov chain steps and 10,000 demerization steps (Appendix D)). We further tested genotypic linkage disequilibrium in GENEPOP, again using Fisher's exact test with 1,000 demerization steps (Raymond and Rousset 1995, Appendix E). A Bonferroni correction was applied to these tests to derive valid confidence intervals (Sokal 1987). We tested for the presence of null alleles using MICROCHECKER (van Oosterhout et al 2004), and adjusted genotypes according to the Oosterhout correction. We estimated effective population sizes using LDNe 1.31 (Waples 2006, Appendix F). We determined the number of total and unique multilocus genotypes within populations and across all individuals using Genalex 6.2 (Peakall and Smouse 2004).

Genetic structure

A model-based Bayesian clustering method was applied to all individual haplotypes using STRUCTURE software (Pritchard and Wen 2004, Falush et al 2003, Pritchard et al 2000) to determine a priori groupings of populations. In this analysis individuals are probabilistically assigned to either a single cluster (the population of origin), or more than one cluster (if there is genetic admixture). The program assumes the neutral unlinked markers to be in Hardy-Weinberg equilibrium and linkage equilibrium and that recent migration would likely produce departures from Hardy-Weinberg equilibrium and linkage equilibrium. STRUCTURE identifies the *K* unknown populations (genetic clusters) of origin of individuals and concurrently allocates all individuals to populations, giving their 90% confidence intervals. STRUCTURE was run using the 'admixture model' and correlated allele frequencies, with a burn-in period of 10,000, followed by 100,000 iterations. Under the assumption that the sampled plants

belong to an unknown number of *K* genetically distinct clusters, we used priors from 1 to 22 to estimate the average posterior probability values for *K* (log likelihood; ln L) for 20 runs each. We found K=20 to be the number of clusters with the highest probability (Figure 3a). To verify the accurate number of clusters (*K*) we followed the graphical methods and algorithms outlined in Evanno *et al* (2005). This method confirmed *K* =20 as the number of clusters with the highest probability (Figure 3b).

We performed Analysis of Molecular Variance (AMOVA) to determine population genetic structure using Arlequin 3.11 software (Excoffier et al. 2005). The analysis was first run using the 20 population distinctions determined by Bayesian clustering. We then further divided the tested populations into four groups to test the previously reported distinctions between populations in the northern, northeastern and southern geographic distribution (see Figure 1): *Group 1- North center of density:* Airport N, Airport S, Airport S Runway, Airport W, Bidwell Ranch, Stone Ridge; *Group 2- Northeast center of density:* Church, Doe Mill, North Enloe, Schmidbauer E, Schmidbauer SE, Schmidbauer W, Stilson Canyon; *Group 3- South center of density:* Hwy 149 North, Dove Ridge E, Dove Ridge N, Dove Ridge SE, Dove Ridge SW, Dove Ridge W; *Group 3- Geographically outlying population* – Table Mountain; *Group 4- Constructed population:* Wurlitzer. The Wurlitzer site, located 6.58 miles(~10 km) north of its closest BCM neighbor was considered geographically separate from group 1. It was constructed in 1994 with seed source from a now extinct site north of Doe Mill and Schmidbauer E (Rod Macdonald, pers.com.). Table Mountain was also considered as separate as it is located at the top of Table Mountain, 4.49 miles from the nearest known BCM occurrence.

We calculated pairwise population comparison matrices (Fst and Nei's genetic distance) using ARLEQUIN 3.11 and portrayed population genetic structure in tree format (Figure 2) using the SAHN clustering algorithm in NTSYSpc with 20,000 bootstraps (version 2.2 Exeter Software, Setauket, NY). To determine whether each sampled individual could be genetically assigned to any of the 21 sampled occurrences we performed Bayesian assignment tests using GENECLASS2 version 2.0.b (Piry et al 2004).

Isolation by Distance, Gene Flow Barrier Analysis, Genetic Bottleneck Analysis

To test for whether genetic distance was confounded by geographic distance we performed Mantel tests (Smouse et al. 1989) using ARLEQUIN 3.11 software (Excoffier et al. 2005, Figure 5a). To test the effect of a transplanted population at the created Wurlitzer site located ~10 km beyond the boundary of the northernmost edge of the known distribution of BCM we evaluated the relationship between genetic and geographic distances both with and without this population (Figure 5b). Also, to avoid the sensitivity of the Mantel test to small population size, we also ran a further analysis excluding populations with less than 15 sampled individuals (all airport sites and Schmidbauer SE; Figure 5c). Further, to assess the presence and location of significant gene flow barriers we employed Monmonier's algorithm using BARRIER 2.2 software (Manni et al 2004). To test for evidence of recent genetic bottlenecks in BCM populations we employed Wilcoxon sign rank tests in BOTTLENECK v. 1.2.02 (Piry et al 1999).

Genetic diversity.

We determined genetic diversity statistics using both ARLEQUIN 3.11 and GENALEX 6.2 software (Peakall and Smouse 2006). We calculated a number of genetic diversity indices per locus across all individuals such as average number of alleles, observed and expected heterozygosities, and Shannon's Information and Fixation Indices (Table 3). For each population we further determined allelic richness corrected for the variation in samples size using FSTAT (Goudet 1995) and the number and percent of fixed loci (Table 4b), and determined the frequencies of private alleles (Table 5).

RESULTS

Genetic Diversity

Limnanthes floccosa ssp. *californica* showed relatively low average genetic diversity within and among individuals at all extant populations (average N alleles per locus = 1.86 ± 0.068 (SE); average H obs = 0.10 ± 0.018 (SE), average H exp = 0.19 ± 0.015 (SE), mean Shannon's information index 0.317 ± 0.025 (SE), mean fixation index 0.556 ± 0.044 (SE), Table 3). This low genetic diversity and decreased level of heterozygosity imply very low natural gene flow via out-crossing. The percentage of

fixed loci per population varied from 11% to 89% (Table 4b, Appendix C). Ten populations exhibited one to three private alleles at frequencies ranging between 0.020 and 0.286 (Table 5).

The most genetically depauperate populations, having five or more fixed loci and low average numbers of alleles per locus were Airport west, south, and north, and Dove Ridge North, followed by Airport south runway, Dove Ridge southwest, Dove Ridge west, Dove Ridge east and Bidwell Ranch (Table 4). The four Airport sites were also those with the smallest population sizes (all below 110 in 2008). The population with the highest average allelic richness and the most private or unique alleles was Hwy 149 North (3 novel alleles, Table 5), rendering it the population with the highest adaptive potential. As such Hwy 149 N is closely followed by Church, North Enloe and Table Mountain (Table 4, high relative allelic richness, and 2 novel alleles each, Table 5). There was one private allele each at Schmidbauer East and Schmidbauer West, and at the genetically depauperate sites: Airport north, Dove Ridge north, Dove Ridge southwest, and Dove Ridge southeast (Tables 4 & 5).

Genetic Structure

Bayesian ordination using STRUCTURE software distinguished 20 distinct population clusters and showed twelve of twenty clusters with fairly homogeneous genetic make-up of individuals (Figure 4a, four airport sites, four DRCB sites, Hwy149N, Stone Ridge, Stilson Canyon Rd., Table Mountain, and Wurlitzer). The analysis indicated the southeastern and southwestern occurrences at Dove Ridge Conservation Bank (DRCB) as a single fairly homogeneous population cluster (Figure 4a). The remaining eight clusters showed populations with a larger proportion of individuals with mixed ancestry (Bidwell Ranch, Church, North Enloe, Doe Mill, and the three Schmidbauer sites), suggesting a larger degree of genetic mixing across populations within the northern geographic distribution of extant BCM sites.

We also show the Bayesian ordination results assuming only 4 population clusters (Figure 4b) to investigate the patterns of genetic structure according to the three centers of population density and outlying populations (Figure 1). The four resulting clusters only marginally reflect these centers of density. The north (Airport N, S, W SR, Stone

Ridge & Bidwell Ranch) and northeast (Church, North Enloe, Stilson Canyon, Doe Mill, Schmidbauer N, W, E, SE) density centers are not clearly delineated in the Bayesian ordination and show some degree of admixture among populations from both centers of density (Figure 4b). Airport North & West are aligned with a portion of individuals from Bidwell Ranch, Church, North Enloe and Stilson Canyon (Figure 4b, red). Airport South and South runway are clustered with Stone Ridge, all Schmidbauer E, SE, & W, Doe Mill and some individuals at Bidwell Ranch, as well as the outlying population at Table Mountain (Figure 4b, yellow). The southern population center is more clearly distinguished from the northern centers, but also shows two groupings, mainly between Dove Ridge Conservation Bank (DRCB) populations (N, SE & SW, Figure 4b, blue) and Hwy 149 North, DRCB West, and the outlying population at Wurlitzer (Figure 4b, green). Individuals at DRCB East show admixture with the northern centers (Figure 4b, red portion).

AMOVA confirmed high population genetic variation and structure among all populations defined via Bayesian ordination ($F_{st} = 0.65$, $P < 0.0000$), explaining 65% of the overall variation, leaving 29% of the variation explained among individuals within populations, and only 5.8% within individuals ($F_{is} = 0.82$, $P < 0.0000$, $F_{it} = 0.94$, $P < 0.0000$; Table 5a). Overall, regional genetic variation or the variation among three centers of density and two outlying populations accounts for 21% of all variation ($F_{ct} = 0.21$, $F_{is} = 0.83$; $F_{sc} = 0.56$, $F_{it} = 0.94$; $P < 0.0000$, Table 6), confirming earlier accounts of such distinct centers of density in this species (Dole and Sun 1992). However, regional groupings explained less of the variation than among population variation within regions, supporting the genetic distinction of individual populations over regional groupings.

Assignment tests investigating the probability that each sampled individual could be assigned to any of the 21 tested occurrences due to their genetic profile overwhelmingly assigned each of the 357 tested individuals to their population of origin. Evidence for populations having undergone a genetic bottleneck (a temporary decline in population size causing the loss of genetic variation) existed only for two tested populations under the step-wise-mutation model: Doe Mill ($P = 0.015$), and Schmidbauer east ($P = 0.055$).

Population cluster analysis using the SAHN algorithm to determine population similarity using Fst (Table 7, Wright 1943) and Nei's genetic distance (Nei 1972) matrices further strongly supported population genetic structure and to a lesser degree geographically distinct centers of population density (Figure 2a & b). Both trees showed close genetic similarity of six populations, all belonging to the northern and northeastern centers of distribution near Chico: Doe Mill, Schmidbauer west, Schmidbauer east, Schmidbauer southeast, Bidwell Ranch, and Stone Ridge. Another group of populations with genetic similarity in both trees were: Church, North Enloe, Stilson Canyon, Airport south runway, and Dove Ridge East. Yet there was no clear geographical or regional affiliation, with most sites in the northeastern, one in the northern and one in the southern center of density. Church and North Enloe, and also Stilson Canyon, being neighboring sites were genetically close to each other, as were Doe Mill and Schmidbauer west, east and southeast, populations all geographically adjacent to each other. The seeded site at Wurlitzer was surprisingly not associated with Doe Mill or Schmidbauer east (the neighboring sites north of which the original seed inoculum was collected), but rather most closely grouped with another mitigation site in the southern region: Hwy 149 North (DR).

The tree based on Nei's genetic distance showed a clearer distinction between the northern/northeastern and the southern distribution centers (excluding Dove Ridge East, yet including the Wurlitzer site) with a genetic distance of 0.66 for both clusters (Figure 2a). Such a regional division was also present but not as clearly resolved in the tree based on Fst (Figure 2b). The Table Mountain site, located on a mountain/mesa top in the southern region, clustered with the northern/northeastern centers of density in both trees, yet represented one of the most genetically distinct sites within this grouping (Figure 2, Table 7). One southern population, Dove Ridge east, consistently clustered with populations of the northeastern and northern region and was most similar to Airport south runway and North Enloe (Fst = 0.175 and 0.157 respectively, Table 7), and genetically distinct from its immediate neighboring populations (Dove Ridge southeast, southwest, north, and west, and Hwy 149 North, Fst > 0.35).

Gene Flow

There is evidence for adequate levels of gene flow between populations that are relatively close to each other geographically throughout the northeastern distribution center within Chico city limits (Doe Mill, Schmidbauer west, east & southeast, and Church, North Enloe and Stilson Canyon, Bidwell Ranch (all similar to each other at varying degrees, Figures 2a & b, Table 7). These genetic similarities were supported by the absence of gene flow barriers within the northeastern distribution center (Figure 6). Gene flow barrier analysis showed six notable barriers (Figure 6). The strongest barrier to gene flow exists between Table Mountain and its closest neighbor population at Hwy 149 North (genetic distance D = 1.057) and can most likely be explained by isolation by distance (IBD). Gene flow between Dove Ridge Conservation Bank (DRCB) west, the northernmost population in the southern distribution center, is effectively cut off from Schmidbauer southeast, the southernmost population in the northeastern center of distribution (D = 0.934), again, a likely function of IBD. Within the southern center of distribution there is an effective gene flow barrier between DRCB east and southeast (D = 0.684), DRCB west and east (D = 0.389), and DRCB southeast and southwest (0.317). Similarly, in the southern center Hwy 149 North is effectively cut off from DRCB east across Hwy 149 (D = 0.594) and DRCB north (D = 0.374). At the northern distribution edge the created Wurlitzer population is isolated from its closest neighboring population Airport north (D = 0.457). Also within the northern center, there exists a barrier to gene flow between Stone Ridge Preserve and Airport south (D = 0.446), and between Airport west and Airport south runway (D = 0.359).

Our results show support for isolation by distance: associating all genetic distances with geographic distances (via a Mantel test) showed a significant positive correlation ($R^2 = 0.22$, P = 0.01, Figure 5a). This correlation slightly increased when the constructed Wurlitzer population was excluded from analysis ($R^2 = 0.25$, P = 0.02, Figure 5b). Since the Mantel test may be sensitive to small population sizes we also evaluated isolation by distance excluding all populations smaller than 15 individuals, showing a significant yet slightly reduced positive correlation ($R^2 = 0.16$, P = 0.01, Figure 5c).

Discussion

Vernal pool ecosystems are in decline throughout California, mainly due to encroachment from urban development, the threats posed by non-native plant competition and other factors that degrade or destroy vernal pool habitat. Development pressure in Butte County's Chico vicinity is mounting and a Butte County Habitat Conservation Plan/Natural Community Conservation Plan (HCP/NCCP) process is under way to direct development activities and so protect the many precious natural resources of the region (BCHCP 2007). As in other areas in northern California the regional vernal pool flora and fauna is at risk (Ayres and Sloop 2008), and the endangered Butte county meadowfoam serves as a beacon for this serious threat in Butte County. Critical habitat was designated for this species (16,636 acres, 6,732 hectares), most of which is located on private property within the Butte Regional HCP/NCCP planning area (USFWS 2006a).

Due to its limited range and habitat restrictions one particularly important recovery goal is reducing the threats to BCM to ensure continued survival throughout its range in perpetuity (USFWS 2005). Other Recovery Plan goals for BCM outlined by USFWS (2005) include studying the method of pollination, protecting habitat for pollinators if necessary, to protect the long-term reproduction, and biosystematic (DNA) research as carried out here. Specific recovery criteria for Butte County meadowfoam include: 1) Protect 100% of known and newly discovered occurrences and 100% of reintroduced occurrences; 2) Protect 95% of suitable habitat within Chico, Doe Mill, Oroville, and Vina Plains within the northeast Sacramento Valley vernal pool region; 3) Reintroduce appropriate races to soil types to replace extirpated occurrences; and 4) Collect seeds for banking within each population (USFWS 2005).

While habitat conditions are at the top of the list of considerations for species recovery, genetic factors are equally important when considering small, isolated and declining populations. Small, less genetically diverse populations are threatened with extirpation from random events, such as extreme weather, as they are less likely to adapt and survive environmental change, even relatively minor events (Frankham et al. 2007). Once small populations decline to a certain point they may enter an 'extinction vortex' where reproductive dynamics such as for example a lack of a critical population number

to attract pollinators via a showy floral display, will favor inbreeding, decreasing effective population sizes and causing even further and often irreversible decline of populations that may eventually result in the extinction of the entire species (Lande 1993). Sufficient genetic diversity within populations maintained by adequate local gene flow can buffer against these detrimental dynamics.

Natural gene flow from neighboring populations usually increases population genetic variation and so heightens the genetic resilience or adaptive potential of populations to avoid severe decline due to catastrophic shifts in environmental conditions. In the absence of adequate gene flow, genetic drift and the effects of inbreeding will cause small, isolated vernal pool plant populations to diverge genetically, especially those that predominantly self-fertilize as is the case with BCM. This reduces within population genetic variation over time and in turn increases the distinction between populations or regional population genetic structure, heightening the severe risk for further decline of a population over time (Elam 1998).

Small population size has been identified as a problem for Butte County meadowfoam at some occurrences, in particular at the four airport occurrences. In 2008 population sizes of these four populations were at fewer than 100 plants and another seven extant populations were at above 100 but fewer than 1000 plants (Table 1). Our results showed high among population genetic structure (Figure 3, Table 6a), and low levels of within population and within individual genetic variation, especially in small extant populations (Table 3, 4b). This suggests that BCM populations are primarily setting seed via self-fertilization, and that the small, declining populations may be heading toward an extinction vortex, rapidly propelling them toward further decline. In the increasingly fragmented and degraded vernal pool system in Butte County, the loss of genetic diversity in the remaining populations of BCM due to inbreeding is no surprise, and leads us to accept our initial hypothesis: Due to apparent predominance of inbreeding throughout the species' range, and the evidently reduced natural gene flow in BCM, low levels of genetic diversity, and substantial genetic structure exist. The challenge now is to find an appropriate mechanism to counter this trend of reduced genetic variation within populations without further endangering declining populations.

While restoring adequate levels of gene flow can generally help to prevent population decline caused by genetic drift and/or inbreeding depression, it can in fact further reduce genetic resiliency in a target population if gene flow comes from a genetically depauperate source (Elam 1998). Further, outbreeding depression (Barrett and Kohn 1991) can occur with the consequence of reducing local adaptation potential (e.g. to microclimate or soil conditions) of a population (e.g. via the breaking up of co-adapted gene complexes) by introducing genetic material too distinct from the recipient population, that if expressed will in turn have negative effects on population survival due to natural selection (Elam 1998). For example, vernal pool soils are variable and are strong agents of selection in these short lived plants, as are competitive interactions within these unique habitats. If source populations are adapted to different conditions than recipient populations such human mediated gene flow supplements may indeed backfire. Consideration of these dynamics during the mixing and/or translocations of seed across the species range for conservation purposes are thus imperative for successful population restoration and recovery. Genetic information should therefore be coupled with detailed information on site microhabitat conditions when determining appropriate seed source sites.

The two main mechanisms for natural gene flow to occur are either seed or pollen dispersal. Overall, BCM is poorly equipped for significant natural gene flow by seed dispersal to other sites (USFWS 2006b), as nutlet dispersal occurs by water, but mostly only short distances (Hauptli et al. 1978). Whether long-distance dispersal via birds is occurring has not yet been established for BCM, but has been suggested for other vernal pool annual plants, such as Baker's sticky seed (*Blennosperma bakeri*). Pollen mediated gene flow also seems limited in BCM because of the high rate of inbreeding, and because of a possible decline of pollinator species due to 1) habitat fragmentation, limiting effective pollinator movement, and 2) the loss of upland habitat that supports pollinators, which have been documented throughout California (Davis 1998; Leong 1994; Thorp and Leong 1995; Thorp and Leong 1998).

Our results identified several notable gene flow barriers between populations, some of which may be due the effects of isolation by distance (Barriers a, b & e, Figure 6), others are likely due to habitat fragmentation via Hwy 149 (Barrier d, Figure 6) or

Chico airport (Barrier f, Figure 6), and some may perhaps be due to a combination of isolation by distance and the loss of effective pollinators connecting the geographically separated populations within Dove Ridge Conservation Bank (Barrier c, Figure 6). Our data also indicated more effective gene flow within the northeastern center of density, where no gene flow barriers were detected perhaps indicating increased out-crossing due to pollinator availability in this center of distribution.

In other species of vernal pool *Limnanthes* solitary bees play an important role in pollination, and these bees usually have a close co-evolutionary relationship with the flowers they pollinate (Thorp 1990; Thorp and Leong 1998). Yet, to what degree BCM depends only on solitary bees for pollination is unclear. Moths, flies, beetles, and other bee species (e.g. honey bees) may also play an important role in pollen dispersal (Mason 1952; Thorp and Leong 1998) but the specific pollinators, pollinator range, and determination of the pollination ecology for BCM has not been determined (USFWS 2005), yet is a highly important topic of future study as identified in the draft HCP/NCCP (BCHCP 2007). Estimation of effective pollen dispersal distances with this potential array of pollinators is a daunting task, yet at the moment a complete lack of effective pollinators may be a more immediate cause for population decline in this species. Habitat loss within the range of BCM is also likely to represent a loss of habitat for its pollinators, but its extent and its effect on the species have yet to be evaluated (USFWS 2005).

The breeding system of BCM is mixed, allowing the species to self-pollinate in the absence of suitable pollinators and effectively outcross in their presence. This is a beneficial strategy in times when pollinators are unavailable, but can become detrimental when pollinators become extinct, as then one mechanism of gene flow ceases. Again, as previously reported by Dole and Sun (1992), our results confirm high rates of inbreeding in BCM. In the absence of effective out-crossing, and seed dispersal (followed by at least some level of out-crossing involving genetic recombination to infuse the newly acquired genetic information into the population) extant populations are thus unable to counteract the effects of genetic drift and inbreeding depression, and continual adaptation of a population to environmental change is severely hindered. Therefore, the recovery of

effective pollinators to allow sexual recombination to counteract inbreeding depression may play a decisive role in the long-term persistence of BCM.

Annual population size fluctuations, breeding system (likely fluctuating geographically and temporally depending on the presence/absence of effective pollinators), and variable yearly seed bank contributions can all affect genetic variation at a given time in a given place. Based on a single measurement of genetic variation, it is thus hard to determine whether genetic variation is stable, increasing or decreasing. At present only populations on some conservation lands are demographically surveyed each year for population sizes as well as estimates of survival and fecundity (Rod Macdonald, pers. com.). As with other annual plants depending on soil seed bank, population sizes for Butte County meadowfoam fluctuate annually and are not necessarily the same at all sites in any given year. Population size as well as the genetic make up of each generation (according to the annual environmental cues that determine some plants to germinate over others) are determined by soil and topography and site-specific interactions with the amount and timing of rainfall, which also influences the average number of annual flowers and nutlets per plant (Dole 1998; Dole and Sun 1992). The largest populations of BCM generally produce the greatest number of nutlets per plant; yet the number of flowers per plant is reduced in dense colonies and the competition from other plant species also reduces flower production (Crompton 1993). All these factors have to be considered in conjunction with the genetic information supplied here to ensure the long-term recovery of individual populations.

Long-term range-wide (including all extant populations) demographic monitoring would be very beneficial to determine the temporal size fluctuations of extant populations and would help identify populations in decline for restoration. Such long-term investigations are important since seed bank stores may adequately buffer populations of annual vernal pool plants from the detrimental dynamics of short-term population decline. Yet this is only the case if the size and genetic diversity of the seed bank remain adequately high over time. As with other vernal pool annuals we can assume that some seeds have remained in the soil for at least the last decade, while others germinated relatively quicker. Plants from such ‘historic’ seeds must have represented a proportion of the plants sampled in this study, representing historic gene flow that can increase the

effective population size in a given year just like contemporary gene flow, and may so equally return important genetic diversity into the population upon outcrossing. Further genetic assays to determine seed bank genetic variation will help us better understand the importance of extant population's adaptive potential stored therein.

We need to determine whether and what level of seed (or pollen) movement by human activities would be beneficial for the recovery of all extant populations, especially those at low population size. In one instance at the Wurlitzer site a substantial number of seed has been moved to inoculate a newly created site, which after more than a decade was genetically divergent from several populations in its historic range that were genetically similar to each other (Doe Mill, Schmidbauer west, east & southeast, and Church, North Enloe and Stilson Canyon, and also Airport north, west, and south runway). After ten years in isolation this population more closely genetically resembled another population (Hwy 149 North) on a mitigation site in the opposite end of the range (Figures 2a & b, Table 7). While this population clearly shows the feasibility of introducing seed inoculum to a previously unoccupied site outside the natural range of the species, we need to evaluate the feasibility and effectiveness of human induced gene-flow into declining populations. Small scale greenhouse trials may allow the evaluation of cross-pollination trials between individuals grown from seeds from a declining site and those originating from potential source sites to determine its feasibility. Cross-pollinated seeds from such trials may then be returned to the site needing to be restored, noting relative survival *in situ*.

While the earlier isozyme study (Dole and Sun 1992) also predicted low levels of genetic diversity and high genetic structure in BCM, 28 isozyme loci could not adequately produce individual genetic fingerprints to effectively evaluate levels of genetic diversity and structure in BCM. Natural selection constrains isozymes to maintain function, making them a more conservative measure of genetic variation, while microsatellites are mainly found in non-functional sections of the genome and so are likely better suited to uncover individual genetic fingerprints. Generally, microsatellite diversity is reported as greater than allozyme diversity (Freville et al 2001, Awadalla and Ritland 1997). We were able to significantly increase the individual level genetic resolution from five allozyme multilocus genotypes in Dole and Sun (1992) to 304 total

and 247 distinct SSR multilocus genotypes using this polymorphic marker system (Table 2). Utilizing only polymorphic markers in our analysis likely inflated our estimates of overall allelic richness and heterozygosity in the species, but it allowed us to more precisely determine the relative amounts of individual versus population genetic variation within BCM. We found population genetic diversity of extant populations of BCM to be low with total number of alleles per locus across nine polymorphic SSR loci ranging between 3 and 8 (Table 4). In contrast, in the equally endangered, yet obligately out-crossing vernal pool congener *Limnanthes vinculans* the number of alleles per locus across 15 loci extended between 9 and 43, and pairwise population Fst values in this species ranged between 0.01 and 0.28 (Ayres and Sloop 2008), as compared to between 0.12 and 0.79 in BCM (Table 7). This is in accord with other studies reporting self-fertilizing populations having lower mean levels of genetic diversity (Gst = 0.191) than obligately cross-fertilizing species (Gst = 0.553; Hamrick and Godt 1996).

Using the population genetic similarity information presented here (Figure 2, Table 7) allows us to infer current gene flow levels and will so help in the daunting task of appropriately re-introducing gene flow to small genetically distinct populations facing inbreeding depression, and to effectively design seed collection strategies for long-term *ex situ* storage. As a rule of thumb, infusion of new alleles should be tested in greenhouse trials, and should be gradual allowing gene flow only between the most similar populations with respect to both genetic distance and habitat similarity. This is to avoid the potential negative effects of outbreeding depression, and to be implemented only once it has been determined without doubt that the population is extremely genetically depauperate, small in size and steadily declining over several years of survey. Our results suggest that candidates for such close examination and potential gene flow re-introduction are: all Airport populations, and all Dove Ridge populations (except for Dove Ridge southeast). It would be extremely prudent to also determine whether effective pollinators exist at all sites, especially at the Airport and Dove Ridge sites, as well as at Bidwell Ranch, since without cross-fertilization the genetic mixing of alleles between the introduced and the recipient individuals will not be accomplished.

To summarize, it seems likely that the patterns of genetic variation and structure we have found are the result of: 1) high rates of inbreeding, 2) significantly reduced

natural dispersal of pollen and seeds across a more and more degraded and fragmented landscape, 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).

Due to the fact that the Butte county vernal pool landscape face threats from urbanization and some small populations have been declining in recent years all remnant populations are to be considered high on the conservation priority list and seed material from all extant populations should be collected over several years for long-term *ex situ* storage and potential future reintroduction. There seems to be effective gene flow between some populations, and there is sufficient evidence that all of the microhabitats should be conserved to maintain the highest possible level of genetic diversity of this naturally rare and endemic species.

The movement of seed to inoculate newly created vernal pools on mitigation banks should be highly regulated, and should take into account the surrounding genetic context of the destination site. Due to the existence of significant isolation by distance, if seed is brought in from a population that is geographically more distant the likelihood of genetic distinction increases. Seed movement to restore declining populations should only occur from source sites that have been genetically tested and after considering all available data on microhabitat, pollinator availability and demography.

The results from this and related future studies should serve to guide seed movement activities, and a working database of the available genetic, and long-term demographic and ecological information (including potential threats) of each extant population should be developed and maintained to inform adequate management of the populations. 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 (http://www.citizen-science.org/). A science advisory panel should be created and consulted to effectively direct this important long-term restoration and conservation process. 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.

Recommendations

The data we presented here are an important part of the needed information to guide this endangered species towards recovery. Threats to the habitat of this species will persist within both protected and unprotected sites, but a better, more detailed knowledgebase, including information on population and pollination ecology and seed bank genetics, and the dynamics of vernal pool ecosystems will help to more effectively guide the restoration and long-term management of the extant populations. To insure species recovery we thus recommend the following investigations and actions:

- To allow a better evaluation of the long-term viability of all populations and their potential for extinction vs. recovery conduct/continue research/surveys into the specific reproductive ecology of BCM investigating:
    - Breeding success (e.g. yearly seed set, viability, germination)
    - Pollination ecology (e.g. dependency on specific pollinators, importance of large contiguous flower displays to attract sufficient pollinator numbers, phenology)
    - Pollinator ecology (of pollinators highly important in successful pollination)
    - Seed dispersal mechanisms (e.g. relative importance of pollen versus seed dispersal)
    - Seed bank dynamics (e.g. size, input, output, genetic variation, germination cues)
    - Conduct further genetic studies to discern genetic variability stored in the seed bank.
- To aid in the recovery of severely declining and genetically depauperate populations implement artificial gene flow after close evaluation of the population status, microhabitat and appropriate seed sources. Small scale greenhouse trials may allow the evaluation of cross-pollination trials between

individuals grown from seeds from a declining site and those originating from potential source sites to determine its feasibility. Cross-pollinated seeds from such trials may then be returned to the site needing to be restored. Generally, the movement of seed inoculum should be conducted in stages and should be highly regulated. It should only occur from source sites that have been genetically tested and are in close microhabitat alignment and relative genetic and geographic proximity to the recipient site. The results from this study should serve as further guidance for seed movement activities.

- Conduct seed collections from all extant sites across several years for *ex situ* long-term storage, and for use in cross pollination trials.
- Implement greenhouse cross-pollination trials to assess appropriate allele sources for declining populations and distribute genetically enriched seeds back into population over several years noting relative survival *in situ*.
- Develop a working database to effectively direct seed inoculation of new and restored sites, and to effectively identify and direct needed restoration and management activities
- Form science-advisory panel to oversee restoration activities
- To ensure appropriate long-term management of the species and its remaining habitat implement detailed micro-habitat surveys and long-term demographic and ecological monitoring of all extant populations using trained volunteer citizen scientists (http://www.citizen-science.org/).

The implementation of these steps will be crucial in realizing the ultimate long-term recovery of this endangered species.

References:

Arroyo, M. 1975. Electrophoretic studies of genetic variation in natural populations of allogamous *Limnanthes alba* and autogamous *Limnanthes floccosa* (Limnanthaceae). Heredity **35:**153-164.

Awadalla, P., and K. Ritland. 1997. Microsatellite variation and evolution in the *Mimulus guttatus* species complex with contrasting mating system. Mol. Ecol. And Evol. **14**: 1023-1034.

Ayers, D., and Sloop, C. 2008. Genetic structure of three endangered plants of the Santa Rosa Plain: Burke's goldfields (*Lasthenia burkei*), Sonoma sunshine (*Blennosperma bakeri*), and Sebastopol meadowfoam (*Limnanthes vinculans*). Grant E-2-P-23 final report submitted to M. Showers, Cal. Dept of Fish and Game, Sacramento.

Barrett, S., and J. Kohn 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. Pages 3-30 in D. Falk and H. K., editors. Conservation of rare plants: biology and genetics. Oxford University Press, Oxford.

BCHCP (Butte County Habitat Conservation Plan). 2007. Administrative Draft Ecological Baseline Conditions Report, May 25, 2007. http://www.buttehcp.com/Document/Admin_Draft_Ecological_Baseline_Report.html

Crompton, K. 1993. Population survey of Butte County meadowfoam (*Limnanthes floccosa* ssp. *californica*), Simmons Ranch, Bruce Road and Humboldt Road, Chico, Butte County California. Unpublished report from Kelley and Associates Environmental Sciences, Chico, CA.

Davis, H. 1998. Allele effects in *Blennosperma bakeri*. San Francisco State University, San Francisco, CA.

Dole, J., and M. Sun 1992. Field and Genetic Survey of the Endangered Butte County Meadowfoam - *Limnanthes floccosa* ssp. *californica* (Limnanthaceae).

Elam, D. 1996. Population genetics of vernal pool plants: theory, data and conservation implications in W. C. E. Al, editor. Ecology, Conservation, and Management of Vernal Pool Ecosystems - Proceedings from a 1996 Conference. California Native Plant Society, Sacramento, CA.

Evanno G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**: 2611-2620.

Excoffier, L., Laval, and S. Schneider 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online. http://cmpg.unibe.ch/softwwere/arlequin3/

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. Genetics **164**: 1567-1587.

Freville, H., F. Justy, and J. Olivieri. 2001. Comparative allozyme and microsatellite populations structure in a narrow endemic plant species, *Centaurea sorymbosa* (Pourret (Asteraceae). Molecular Ecology **10**: 879-889.

Finn, M. 2000. Natural environmental study for State Route 99/149/70. California Department of Transportation, Marysville, CA.

Foin, T., S. Reilly, A. Pawley, D. Ayers, T. Carlson, P. Hodem, and P. Switzer 1998. Improving recovery planning for the conservation of threatened and endangered taxa. Bioscience **48:**177-184.

Futuyama, D. 1986. Evolutionary Biology. Pages 345. Sinauer Associates, Inc., Sunderland, MA.

Frankham, R., J. Ballou, and D. A. Briscoe. 2007. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Goldstein, D. B., and C. Schloetterer (eds.). 1999. Microsatellites – Evolution and Applications. Oxford University Press, Oxford, U.K.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity **86**: 485-486.

Hamrick, J. L., and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. Phil. Trans. R. Soc. Lond. B, **351**: 1291-1298.

Harker, N. 2001. Collection, Reporting and Storage of Microsatellite Genotype Data in R. Henry, editor. Plant Genotyping: the DNA fingerprinting of Plants. CAB International.

Hickman, J. C. (ed.). 1993. The Jepson Manual – Higher Plants of California. Science **262:**261-261.

Holland, R. 1976. The vegetation of vernal pools: a survey. Pages 11-15 in S. Jain, editor. Verbal pools: their ecology and conservation. University of California, Davis.

Holton, T. 2001. Plant Genotyping by Analysis of Microsatellites in R. Henry, editor. Plant Genotyping: the DNA fingerprints of plants. CAB International.

Jokerst, J. 1989. A draft plan for the conservation of Butte County meadowfoam in the City of Chico *Limnanthes floccosa* ssp. *californica*. Prepared for: City of Chico Community Services Department, Chico, CA, Sacramento, CA.

Kishore et al. 2004. Conserved simple sequence repeats for the Limnanthaceae. Theoretical and Applied Genetics **103:**450-457.

Kelley and Associates Environmental Sciences, I. 1994. Population survey of Butte County meadowfoam (*Limnanthes floccosa* ssp. *californica*), Stonegate Project - 1994, Bruce Road and Warfield Road, Chico, Butte County, California, Unpublished report, Davis, California.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. American Naturalist **142:**911-927.

Leong, J. 1994. Pollination of a patchily-distributed plant, Blennosperma. University of California, Davis.

Manni, F., E. Guérard, and E. Heyer 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm". Human Biology **76**: 173-190.

Mason, C. 1952. A systematic study of the genus Limnanthes. Pages 455-512. University of California Publications in Botany.

Nei, M. 1972. Genetic distance between populations. American Naturalist **106**:283-&.

Peakall, R., and P. E. Smouse 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6:**288-295.

Piry S., A. Alapetite, J.-M. Cornuet et al, 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity **95**: 536-539.

Piry S., G. Luikart, J.-M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity **90**: 502-503.

Pritchard, J. K, and W. Wen. 2004. Documentation for Structure Software: Version 2. http://pritch.bsd.uchicago.edu

Pritchard, J. K., M. Stephens, and P. Donelly. 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics **155**: 945-959.

Raymond, M., and F. Rousset. 1995. GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. Journal of Heredity **86**: 248-249.

Rohlf, J. 2005. NTSYSpc Version 2.2. Numerical Taxonomy and Multivariate Analysis System. Exeter Software, Setauket, NY.

Sokal, R. R. 1987. Introduction to Biostatistics. W. H. Freeman & Co.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science **236:**787-792.

Smouse, P. E., J. C. Long, R. R. Sokal. 1986. Multiple regression and correlation extension of the Mantel test of matrix correspondence. Systematic zoology **35**: 627-632.

Thorp, R. 1990. Vernal pool flowers and host-specific bees in D. Ikeda and R. Schlising, editors. Vernal pool plants: their habitat and biology. California State University, Chico, CA.

Thorp, R., and L. Jm 1998. Specialist bee pollinators of showy vernal pool flowers. Ecology, Conservation, and Management of Vernal Pool Ecosystems - Proceedings from a 1996 Conference, Sacramento, CA1998. California Native Plant Society, [insert place of publication].

Thorp, R., and J. Leong 1995. Native bee pollinators of vernal pool plants. Fremontia **23**.

U.S. Fish and Wildlife Service (USFWS) 1992. Endangered and threatened wildlife and plants; determination of endangered status for the plant *Limnanthes flocccosa* ssp. *californica* (Butte County meadowfoam). Pages 24192-24199. Federal Register.

U.S. Fish and Wildlife Service (USFWS) 2005. Recovery plan for the vernal pool ecosystems of California and Southern Oregon. http://www.fws.gov/sacramento/es/recovery_plans/vp_recovery_plan_links.htm

U.S. Fish and Wildlife Service (USFWS) 2006a. Endangered and Threatened Wildlife and Plants: Designation of Critical Habitat for Four Vernal Pool Crustaceans and Eleven Vernal Pool Plants; Final Rule. 50 CFR Part 17. February 10, 2006.

U.S. Fish and Wildlife Service (USFWS) 2006b. Species Account: Butte County meadowfoam (*Limnanthes flocccosa* ssp. *californica*). http://www.fws.gov/sacramento/es/plant_spp_accts/butte_county_meadowfoam.htm

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills et al. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**: 535-538.

Waples, R. S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conservation Genetics **7**:167–184.

Wright, S. 1943. Isolation by distance. Genetics **28:**114-138.

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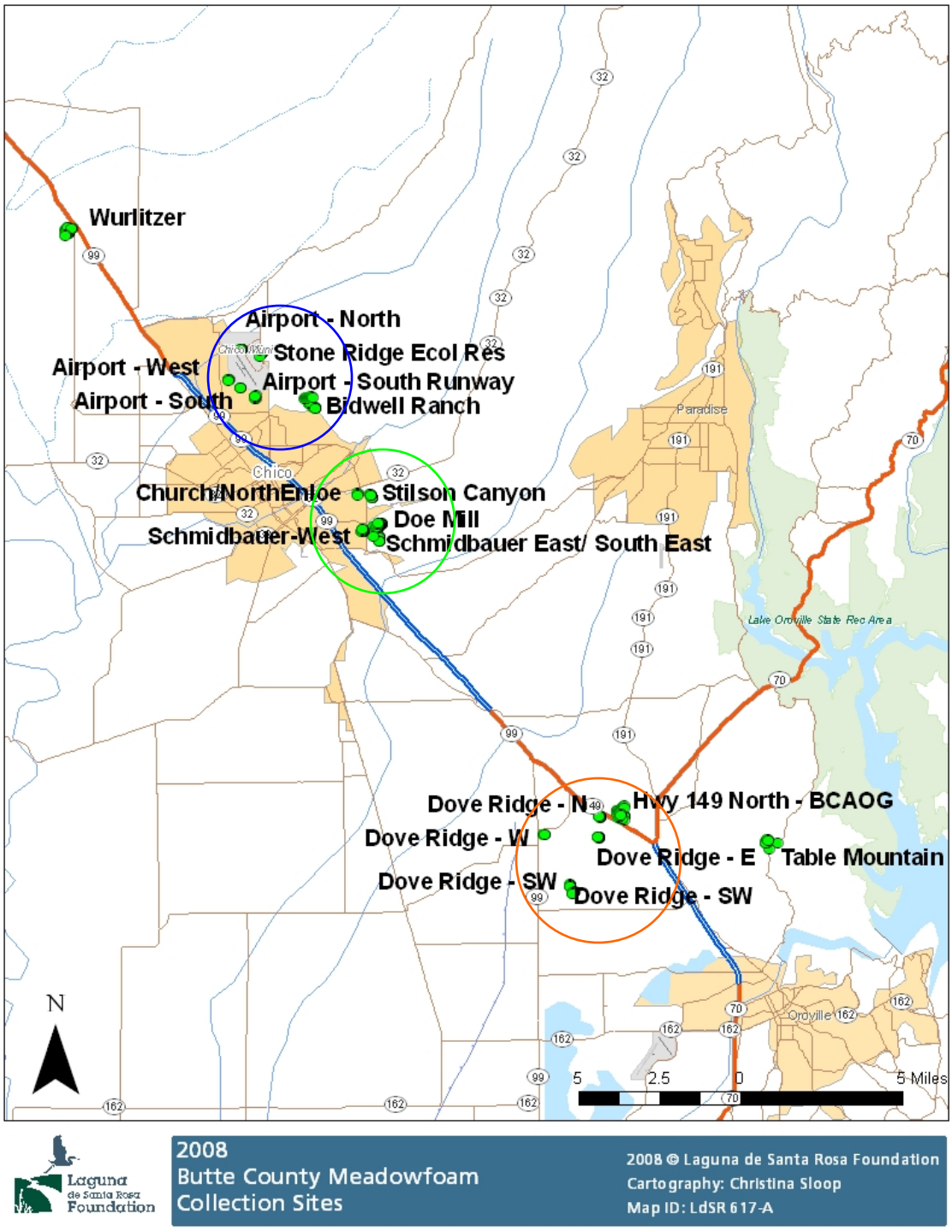

Figure 1: 2008 Butte County Meadowfoam collection sites. Circles depict centers of population density as defined by Dole and Sun (1992): Blue – north; Green – northeast; Red – south. Two remaining outlying occurrences are at Table Mountain in the south and Wurlitzer in the North.

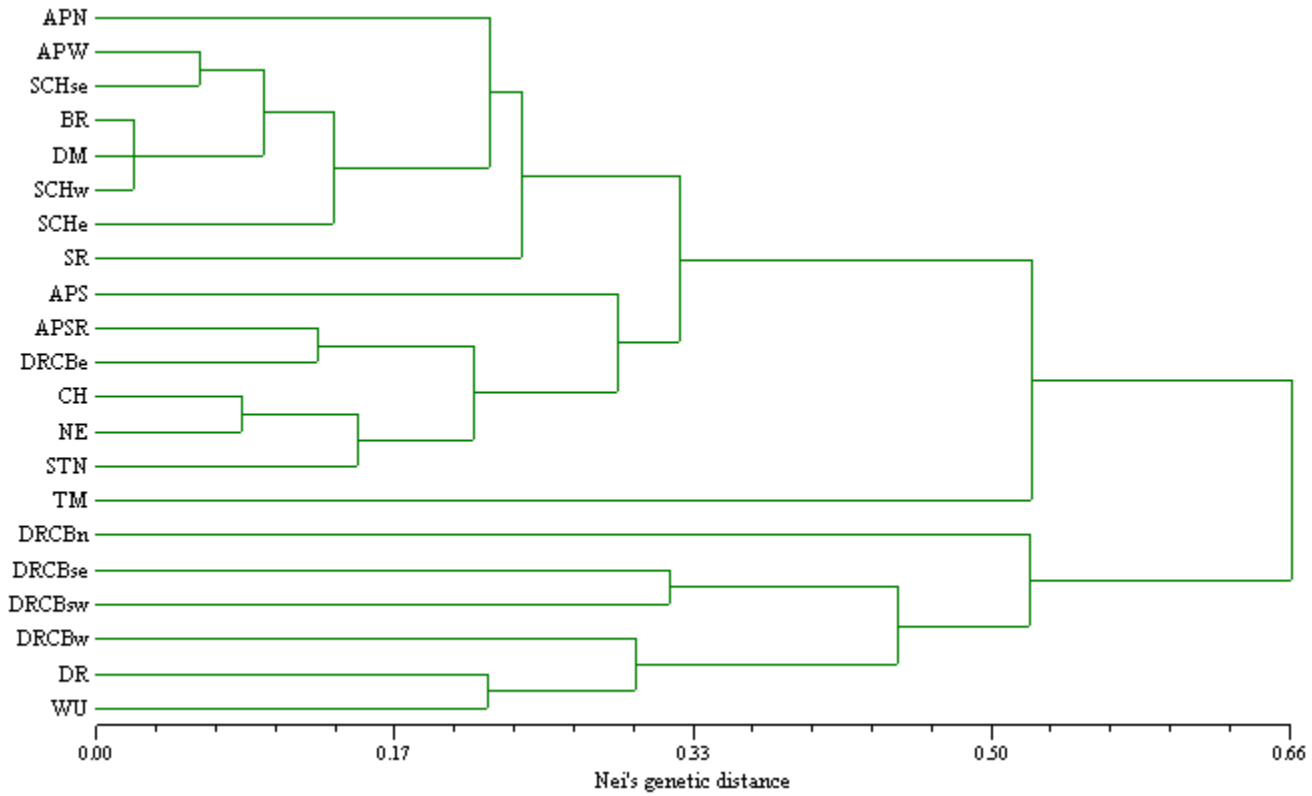

Figure 2a: Population tree based on the SAHN clustering algorithm using Nei's genetic distance (based on 20,000 bootstraps). *APN* – Airport N, *APW* – Airport W, *APS* – Airport S, *APSR* – Airport S Runway, *BR* – Bidwell Ranch, *CH* - Church, *DM* – Doe Mill, *DR* – Hwy 149 N, *DRCBn* – Dove Ridge N, *DRCBw* – Dove Ridge W, *DRCBsw* – Dove Ridge SW, *DRCBe* – Dove Ridge E, *DRCBse* – Dove Ridge SE, *NE* – North Enloe, *SCHe* – Schmidbauer E, *SCHse* – Schmidbauer SE, *SCHw* – Schmidbauer W, *SR* – Stone Ridge, *STN* – Stilson Canyon, *TM* – Table Mountain, *WU* – Wurlitzer.

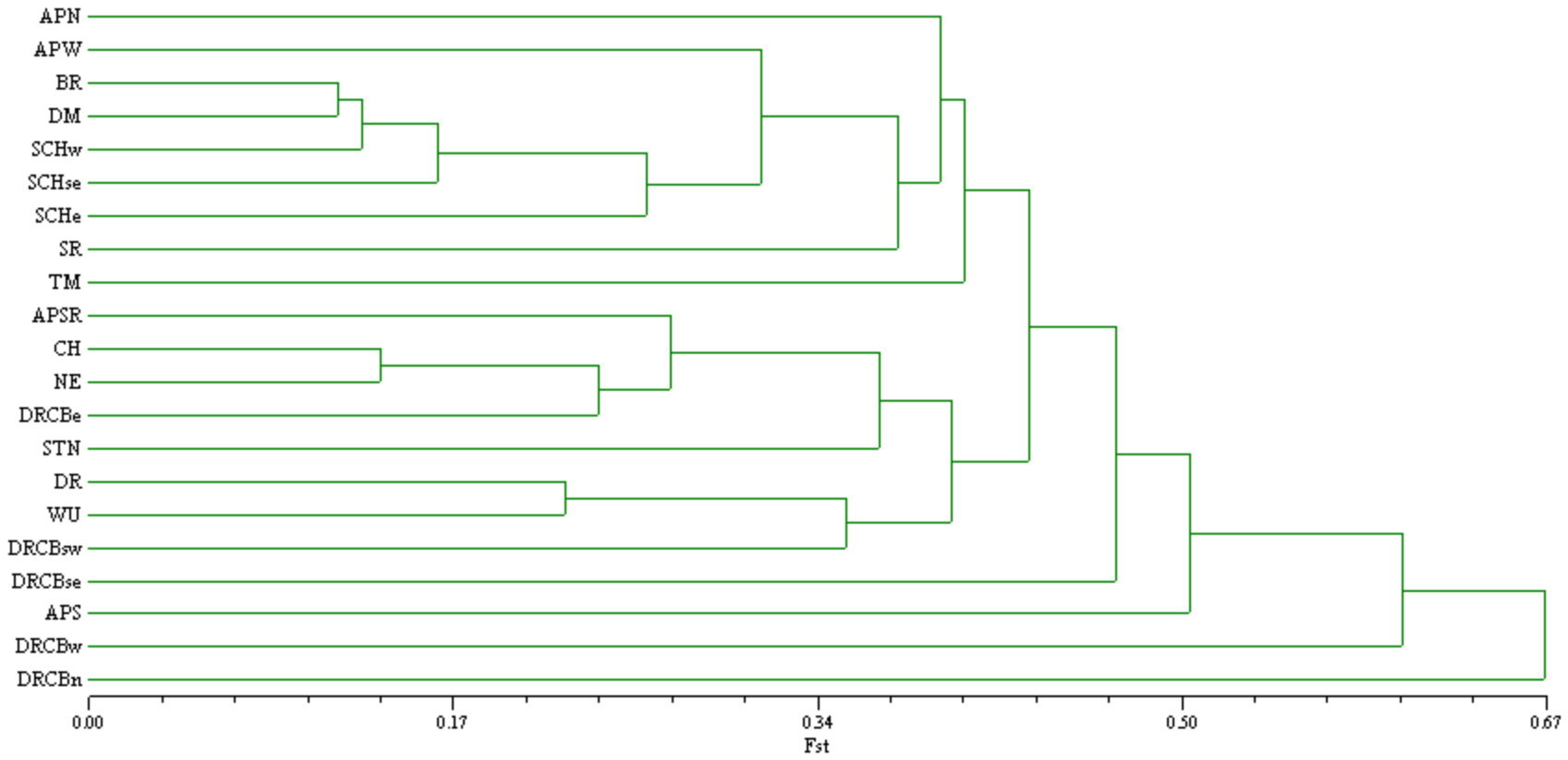

Figure 2b: Population tree based on the SAHN clustering algorithm using a population similarity matrix based on Wright's Fst (based on 20,000 bootstraps). *APN* – Airport N, *APW* – Airport W, *APS* – Airport S, *APSR* – Airport S Runway, *BR* – Bidwell Ranch, *CH* - Church, *DM* – Doe Mill, *DR* – Hwy 149 N, *DRCBn* – Dove Ridge N, *DRCBw* – Dove Ridge W, *DRCBsw* – Dove Ridge SW, *DRCBe* – Dove Ridge E, *DRCBse* – Dove Ridge SE, *NE* – North Enloe, *SCHe* – Schmidbauer E, *SCHse* – Schmidbauer SE, *SCHw* – Schmidbauer W, *SR* – Stone Ridge, *STN* – Stilson Canyon, *TM* – Table Mountain, *WU* – Wurlitzer.

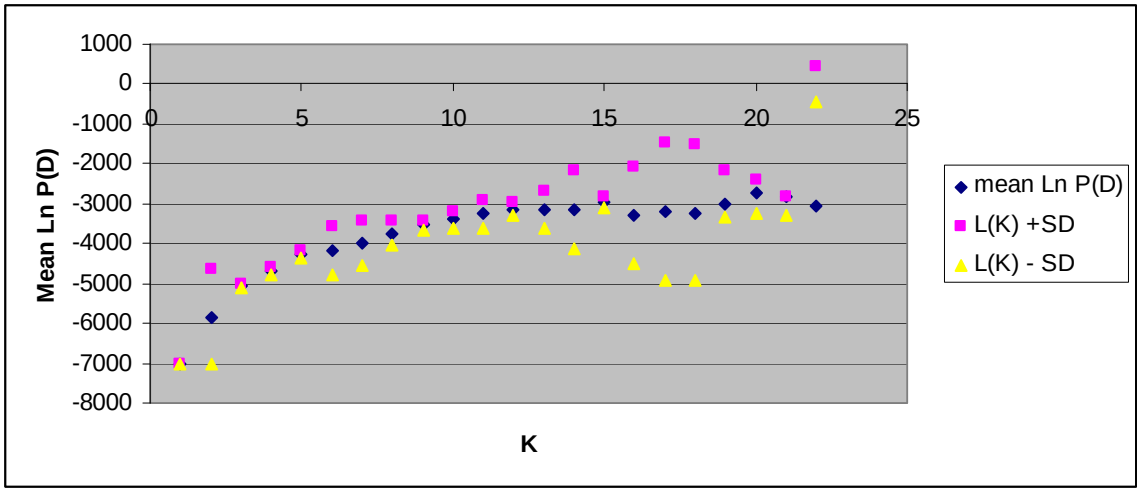

Figure 3a: Mean Ln P(D) for K = 1 through K = 22 averaged across 20 STRUCTURE simulations using the admixture model, a burn-in of 10,000 iterations, and 100,000 iterations for each run.

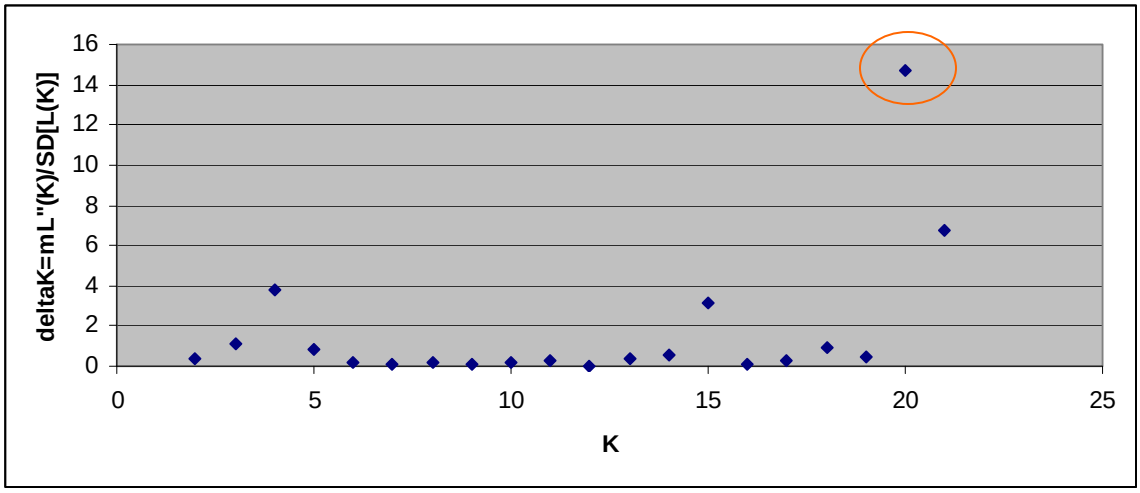

Figure 3b: The most likely number of clusters, determined by the algorithms and graphical methods described in Evanno et al (2005), as K = 20 (circled).

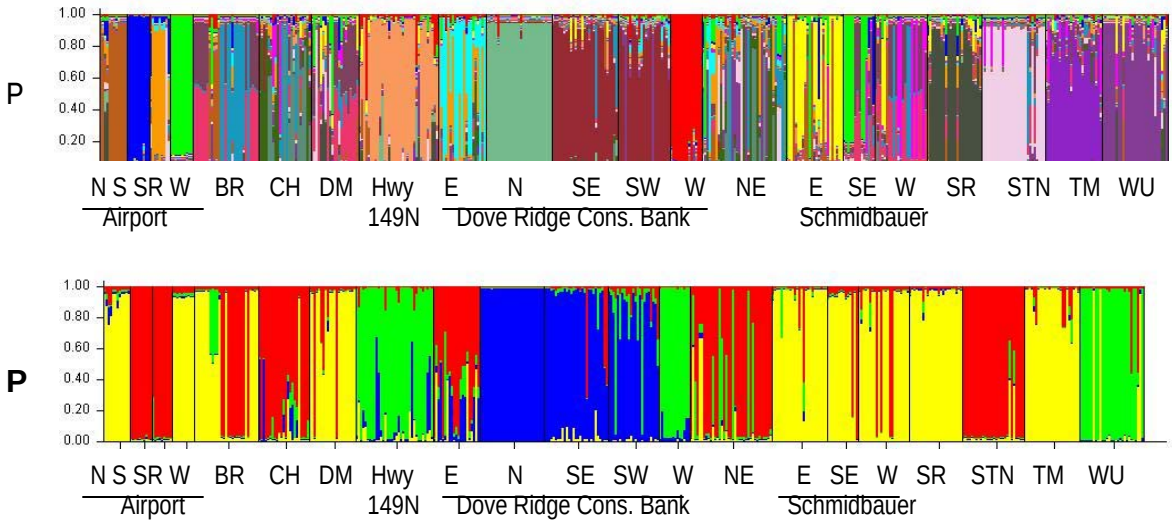

Figure 4: Membership of BCM individuals in **a)** K = 20 **b)** K = 4 population clusters determined via the a priori Bayesian clustering method in STRUCTURE. Each vertical line represents an individual's probability of belonging to one of K clusters (represented by the various colors) or a combination thereof if ancestry is mixed. Site order (left to right): N – Airport north, S – Airport south, SR – Airport south runway, W – Airport W, BR – Bidwell Ranch, CH – Church, DM – Doe Mill, E – Dove Ridge Conservation Bank (DRCB) east, N – DRCB north, SE- DRCB southeast, SW – DRCB southwest, NE – North Enloe, E – Schmidbauer (SCH) east, SE – SCH southeast, W – SCH west, SR – Stone Ridge Preserve, STN – Stilson Canyon Rd., TM – Table Mountain, WU – Wurlitzer.

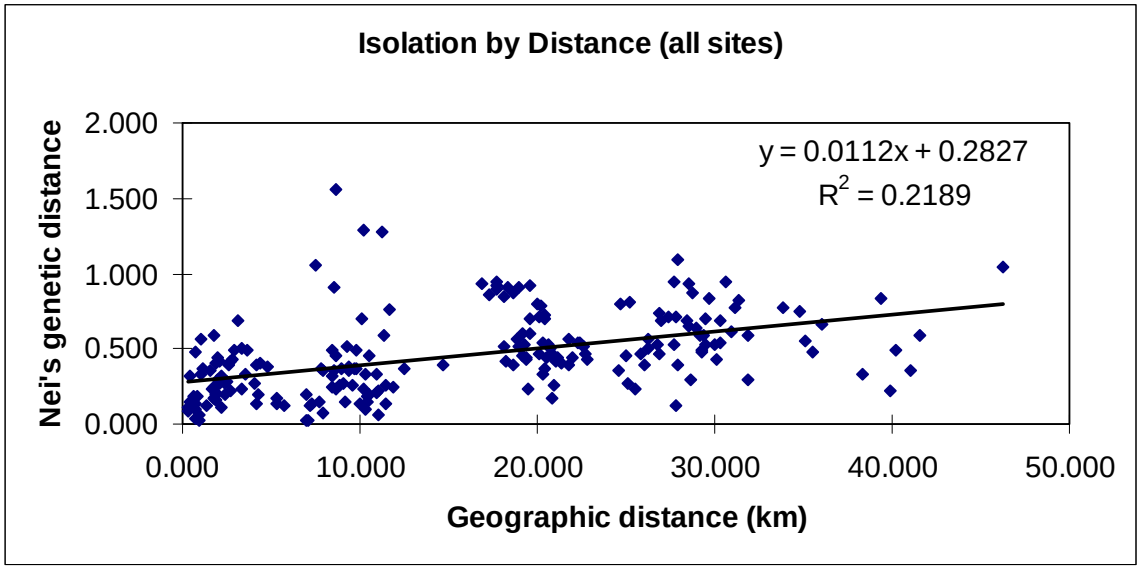

Figure 5a: Correlation between Geographic Distance and Genetic Distance across 20 of 21 sites using a Mantel test (100 permutations, P = 0.01)

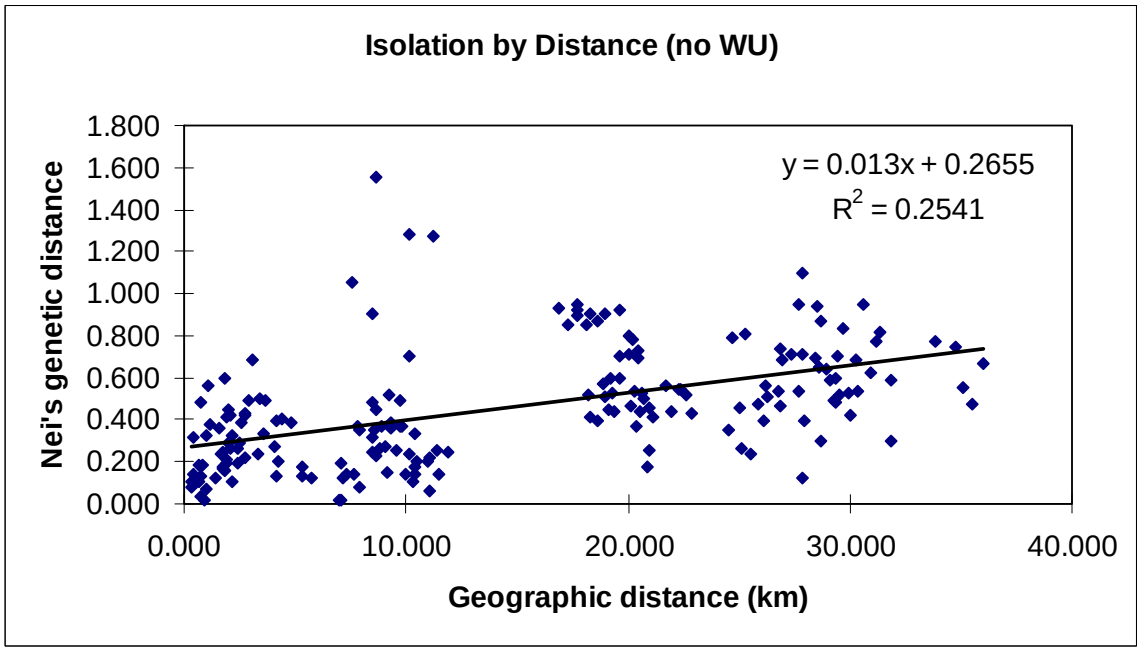

Figure 5b: Correlation between Geographic Distance and Genetic Distance across 20 of 21 sites (excluding the artificially created Wurlitzer site at the northern edge of the distribution) using a Mantel test (100 permutations, P = 0.02).

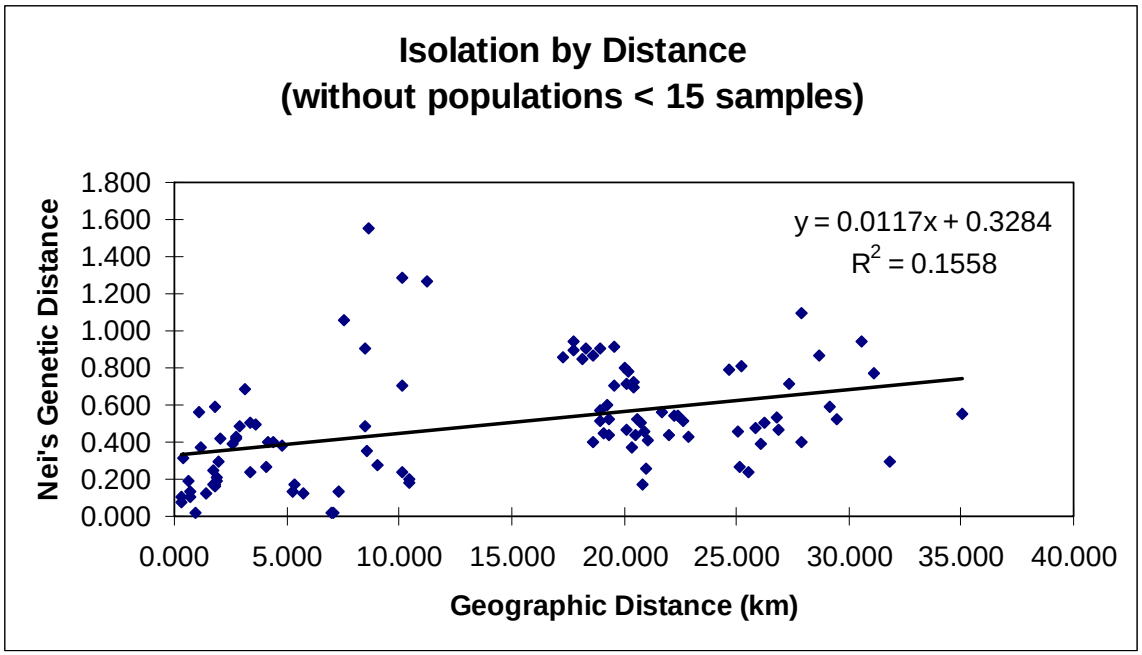

Figure 5c: Correlation between Geographic Distance and Genetic Distance across 15 of 21 sites (excluding all Airport populations & Schmidbauer SE with samples <15 individuals) using a Mantel test (100 permutations, P = 0.01).

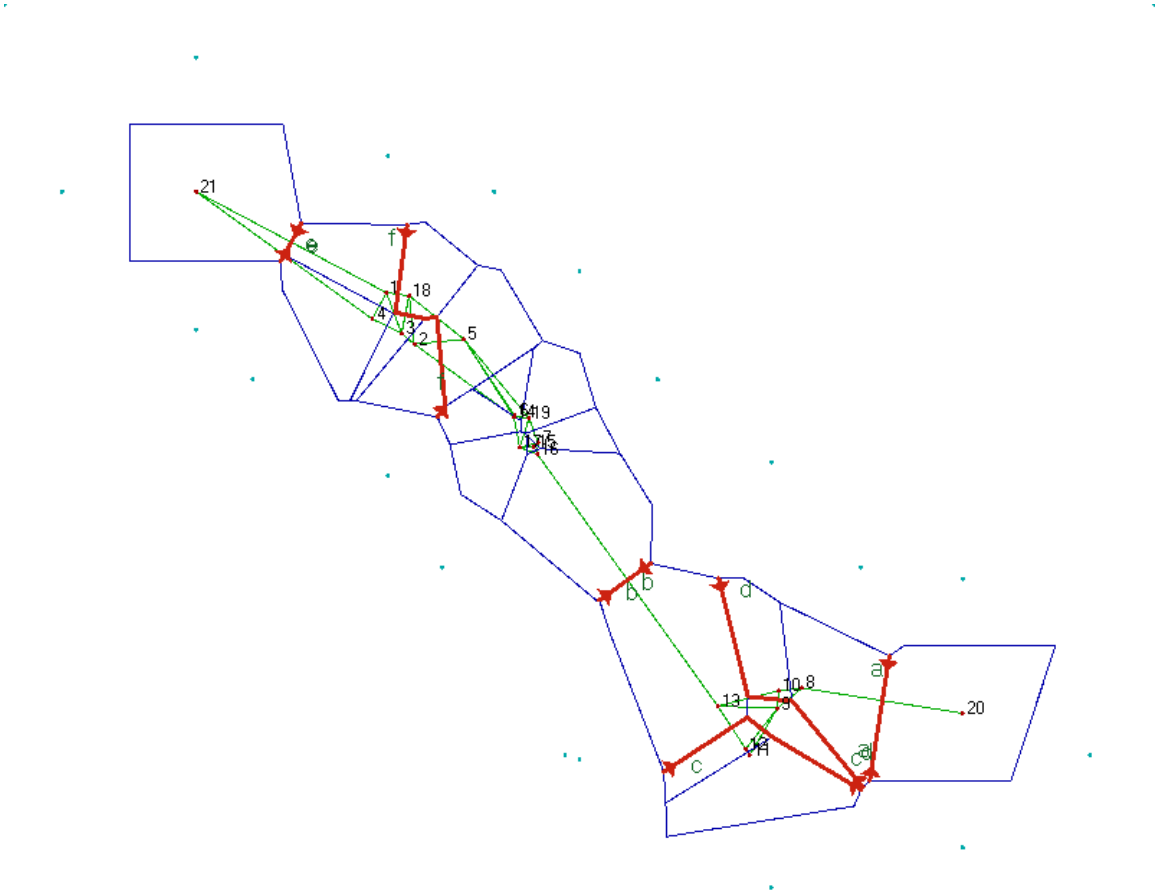

Figure 6: Monmonier's algorithm analysis; map indicating notable gene flow barriers (a-f) between BCM populations based on geographic coordinates and Nei's genetic distance. Barrier a: Table Mountain & Hwy 149 N, Distance (D) = 1.057; Barrier b: Dove Ridge Conservation Bank (DRCB) west & Schmidbauer southeast, D = 0.934; Barrier c: DRCB east and southeast, D = 0.684; Barrier d: Hwy 149N & DRCB east; D = 0.594; Barrier e: Wurlitzer & Airport north, D = 0.457; Barrier f: Stone Ridge Preserve & Airport south, D = 0.446.

Table 1: Summary of 2008 BCM DNA Collections

Coll. Date	Site	Site Code	Distance to nearest BCM site	Population Size Estimate	# DNA Samples ***
3/24/2008	Stone Ridge Ecological Reserve	SR	0.63 mi (APN)	~1000*	40
3/24/2008	Butte Co. Airport - West	APW	0.44 mi (APS)	47	10
3/24/2008	Butte Co. Airport - South	APS	0.44 mi (APW)	36	10
3/25/2008	Butte Co. Airport - North	APN	0.63 mi (SR)	105	12
3/25/2008	Butte Co. Airport - South Runway	APSR	0.51 mi (APS)	~81	10
3/24/2008	Stilson	STN	0.38 mi (CH)	~500*	30
3/2520/08 & 3/27/2008	Bidwell Ranch	BR	1.65 mi (APSR)	~5000*	40
3/26/2008	Wurlitzer	WU	6.58 mi (APN)	6078**	36
3/26/2008	Doe Mill Reserve	DM	0.08 – 0.50 mi (SCH-E) separated by wall & river	~8177**	30
3/26/2008	Church	CH	0.06 – 0.30 mi (NE) separated by road	~1660*	36
3/26/2008	North Enloe	NE	0.06 – 0.30 mi (CH) separated by road	~1065*	40
3/27/2008	Hwy 149 North	DR	0.60 mi (DRCB-N)	~802	46
3/27/2008	Schmidbauer -East	SCH-E	0.08 – 0.50 mi (SCH-E) separated by wall & river	~1365*	26
3/27/2008	Schmidbauer -South East	SCH-SE	0.13 – 0.25 mi (SCH-E) separated by wall	~200*	14
3/27/2008	Schmidbauer -West	SCH-W	0.32 mi (SCH-E)	~452*	25
3/28/2008	Table Mountain	TM	4.49 mi (DR)	~210*	36
4/2/2008	Dove Ridge - North	DRCB-N	0.60 mi (DR)	~365	30
4/2/2008	Dove Ridge - East	DRCB-E	0.67 mi (DRCB-N)	~600	35
4/2/2008	Dove Ridge - SE	DRCB-SE	0.31 mi (DRCB-SW)	~1000	35
4/2/2008	Dove Ridge - SW	DRCB-SW	0.31 mi (DRCB-SE)	~2000	30
4/2/2008	Dove Ridge - W	DRCB-W	1.67 mi (DRCB-E)	~159	15
				Total samples	**586**

*Did not survey entire geographic extent of the site, but only where plants had been located in previous years. **Rod McDonald (Wurlitzer Foundation) surveyed entire population size in 2008. Doe Mill plant numbers estimated from capsule count divided by 2.45 average capsules per plant.
Note: Sample collections were limited to leaf/stem samples unless small size of plant required taking entire individual.

Table 2: Geographical Site Coordinates and Summary of 2008 BCM DNA Collections and Samples Genotyped.

Site	Longitude	Latitude	Site Code	No. Samples Collected	No. Samples Genotyped *	No. Multilocus Genotypes	No. Unique Multilocus Genotypes
Stone Ridge Ecological Reserve	-121.84764788723	39.80263425298	SR	40	24	16	12
Butte Co. Airport - West	-121.86707011933	39.79037473092	APW	10	10	2	1
Butte Co. Airport - South	-121.84493329840	39.77715627728	APS	10	10	6	5
Butte Co. Airport - North	-121.85961912491	39.80425200332	APN	12	12	7	5
Butte Co. Airport - South Runway	-121.85162773678	39.78272263107	APSR	10	9	6	4
Stilson Canyon	-121.78434218915	39.73804975500	STN	30	15	8	5
Bidwell Ranch	-121.81862077329	39.77961561691	BR	40	29	14	9
Wurlitzer	-121.95998109205	39.85743712019	WU	36	29	15	11
Doe Mill Reserve	-121.77959243839	39.72510489105	DM	30	21	18	15
Church	-121.79189128350	39.73934762445	CH	36	23	21	19
North Enloe	-121.79230069900	39.73871417650	NE	40	37	33	30
Hwy 149 North	-121.64027312679	39.59579013414	DR	46	35	31	27
Schmidbauer -East	-121.78168228154	39.72303765924	SCH-E	26	25	20	17
Schmidbauer - South East	-121.77996510209	39.71909314882	SCH-SE	14	14	10	8
Schmidbauer -West	-121.78931211188	39.72271262524	SCH-W	25	23	11	7
Table Mountain	-121.55545780713	39.58248403511	TM	36	25	23	21
Dove Ridge - North	-121.65236646925	39.59430410989	DRCB-N	30	29	12	8
Dove Ridge - East	-121.65327532592	39.58506085639	DRCB-E	35	21	17	14
Dove Ridge - SE	-121.66795844587	39.56035775823	DRCB-SE	35	29	24	20
Dove Ridge - SW	-121.66996014127	39.56358696060	DRCB-SW	30	23	20	17
Dove Ridge - W	-121.68474670617	39.58629692987	DRCB-W	15	14	7	4
			Total	**586**	**457**	**309**	**247**

* Includes individuals with data for a minimum of 7of 9 marker loci.

Table 3: Population Genetic Diversity Indices: Average No. of individuals genotyped per locus (N), No. alleles (Na), No. Effective Alleles (Ne), Shannon's Information Index (I), Observed Heterozygosity (Ho), Expected (He) and Unbiased Expected (UHe) Heterozygosity, and Fixation Index (F), averaged for all individuals per locus and across 9 loci (Total).

	N		**Na**		**Ne**		**I**		**Ho**		**He**		**UHe**		**F**	
Locus	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***
LS527	20.286	*1.745*	1.667	*0.187*	1.275	*0.085*	0.257	*0.068*	0.009	*0.004*	0.158	*0.044*	0.162	*0.045*	0.935	*0.022*
LS02	19.714	*1.769*	1.905	*0.194*	1.313	*0.091*	0.301	*0.068*	0.022	*0.008*	0.180	*0.043*	0.187	*0.045*	0.823	*0.062*
LS166	20.905	*1.744*	1.286	*0.122*	1.085	*0.050*	0.085	*0.042*	0.030	*0.020*	0.052	*0.028*	0.053	*0.029*	0.657	*0.080*
LS43	17.524	*1.856*	1.810	*0.203*	1.275	*0.071*	0.288	*0.066*	0.051	*0.019*	0.172	*0.040*	0.184	*0.045*	0.651	*0.081*
LS122	20.571	*1.674*	1.095	*0.066*	1.050	*0.048*	0.038	*0.033*	0.050	*0.048*	0.026	*0.024*	0.026	*0.024*	-0.511	*0.151*
LS179	18.286	*1.894*	2.714	*0.156*	2.009	*0.075*	0.764	*0.038*	0.676	*0.062*	0.487	*0.021*	0.505	*0.022*	-0.363	*0.105*
LS164	21.048	*1.744*	2.095	*0.181*	1.423	*0.085*	0.419	*0.065*	0.017	*0.006*	0.251	*0.040*	0.256	*0.041*	0.912	*0.029*
LS184	20.476	*1.646*	2.095	*0.206*	1.422	*0.109*	0.386	*0.078*	0.016	*0.006*	0.227	*0.048*	0.232	*0.049*	0.817	*0.079*
LS321	19.048	*1.763*	2.048	*0.263*	1.303	*0.087*	0.315	*0.078*	0.031	*0.018*	0.175	*0.044*	0.182	*0.046*	0.856	*0.048*
Total	**19.762**	***0.581***	**1.857**	***0.068***	**1.351**	***0.032***	**0.317**	***0.025***	**0.100**	***0.018***	**0.192**	***0.015***	**0.199**	***0.016***	**0.556**	***0.044***

Table 4a: Allelic Richness per Locus in *L. alba* (Kishore et al 2004) vs. *L. floccosa* ssp. *californica*

	LS 527	**LS 02**	**LS 166**	**LS 43**	**LS 122**	**LS 179**	**LS 164**	**LS 184**	**LS 321**	**Ave No. alleles (SD)**
Total alleles *L. alba*	10	9	11	10	13	7	10	11	12	**10.3 (1.7)**
Total alleles *L. floccosa* ssp. *californica*	4	6	3	6	3	5	6	6	8	**5.2 (1.6)**

Table 4b: Allelic Richness (corrected for sample size) and No. of Fixed Loci per Locus and Population.

	APN	APS	APSR	APW	BR	CH	DM	Hwy 149	DRCBe	DRCBn	DRCBse	DRCBsw	DRCBw	NE	SCHe	SCHse	SCHw	SR	STN	TM	WU
LS527	1	1	1	1	1	2.173	1	1.785	1.893	1	1.373	1	1	1	1.315	1.697	1.657	1	1.253	1.879	1.397
LS02	1	1	1.971	1	1	2.274	1	1.811	1	1	1.777	1	1.481	1.877	1.265	1.27	1.345	1.27	1.133	1.291	1.695
LS166	1	1	1	1	1	1	1.184	1.174	1	1	1	1	1	1.109	1	1	1	1.783	1	1.799	1
LS43	1	1	1	1	1	1.852	1.478	1.897	1.481	1.675	1.771	1.382	2	1.642	1.41	1	1	1	1	1.577	1
LS122	1	1	1	1	1	1.087	1	1	1	1	1	1	1	1	1	1	1	1	1	1.891	1
LS179	2.171	1.923	1.881	1.918	1.732	2.233	1.962	1.928	2.145	2.295	1.907	1.892	2.435	2.01	1.593	2.039	1.674	1.89	1.816	1.546	2.053
LS164	1.544	1	1	1	1.844	1.372	1.767	2.04	1.606	1	2.172	1.396	1	1.839	1.226	1.382	1.547	1.886	1.454	1.547	1.687
LS184	1	1	1.405	1	1.675	1.095	2	2.186	1	1	1.591	1	1.143	2.321	2.036	1.887	1.482	1.659	1.676	1	1.402
LS321	1.81	1	1.786	1	1.547	1.87	1.202	1.925	1	1	1	2.198	1	1.482	2.02	1	1	1	1	1.235	1.512
Average	1.28	1.10	1.34	1.10	1.31	1.66	1.40	1.75	1.35	1.22	1.51	1.32	1.34	1.59	1.43	1.36	1.30	1.39	1.26	1.53	1.42
St. dev.	0.45	0.31	0.43	0.31	0.38	0.53	0.42	0.40	0.45	0.46	0.44	0.45	0.53	0.47	0.39	0.42	0.30	0.41	0.32	0.31	0.37
# fixed loci	6	8	5	8	5	1	3	1	5	7	3	5	5	2	2	4	4	4	4	1	3
% fixed loci	67%	89%	56%	89%	56%	11%	33%	11%	56%	78%	33%	56%	56%	22%	22%	44%	44%	44%	44%	11%	33%

Table 5: Private allele frequencies per population

Pop	Locus	Allele	Freq
Airport North	LS321	283	0.111
Church	LS43	230	0.023
Church	LS122	399	0.022
Hwy 149 North	LS164	237	0.286
Hwy 149 North	LS184	325	0.015
Hwy 149 North	LS321	287	0.014
Dove Ridge N	LS43	221	0.091
Dove Ridge SE	LS02	185	0.250
Dove Ridge SW	LS321	271	0.059
North Enloe	LS02	198	0.014
North Enloe	LS166	228	0.028
Schmidbauer E	LS02	177	0.071
Schmidbauer W	LS02	197	0.023
Table Mountain	LS122	397	0.500
Table Mountain	LS321	275	0.020

Table 6a: Analysis of Molecular Variance (AMOVA) across 20 populations*:* Airport N, Airport S, Airport S Runway, Airport W, Bidwell Ranch, Stone Ridge; Church, Doe Mill, North Enloe, Schmidbauer E, Schmidbauer SE, Schmidbauer W, Stilson Canyon; Hwy 149 North, Dove Ridge E, Dove Ridge N, Dove Ridge SE/SW, Dove Ridge W; Table Mountain, Wurlitzer.

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations within groups	19	683.370	0.82717 Va*	64.53
Among individuals within populations	436	337.648	0.37290 Vb*	29.09
Within individuals	457	35.000	0.08178 Vc *	5.80
Total	912	1056.018	1.28184	

Significance tests (1023 permutations): * P < 0.0000

Fixation Indices

FIS : 0.82014
FST : 0.64530
FIT : 0.93620

Table 6b: Analysis of Molecular Variance (AMOVA) across three centers of density and two outlying populations; see Fig. 1 for site locations): *Group 1:* Airport N, Airport S, Airport S Runway, Airport W, Bidwell Ranch, Stone Ridge, *Group 2:* Church, Doe Mill, North Enloe, Schmidbauer E, Schmidbauer SE, Schmidbauer W, Stilson Canyon; *Group 3:* Hwy 149 North, Dove Ridge E, Dove Ridge N, Dove Ridge SE/SW, Dove Ridge W; *Group 4:* Table Mountain; *Group 5:* Wurlitzer (constructed site - seed source from extinct site north of Doe Mill, Rod Macdonald, pers.com.).

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	4	312.363	0.28311 Va*	20.85
Among populations within groups	16	416.219	0.60677 Vb*	44.68
Among individuals within populations	436	374.441	0.39056 Vc*	28.76
Within individuals	457	35.500	0.07768 Vd **	5.72
Total	913	1138.523	1.43096	

Significance tests (1023 permutations): * P < 0.0000, P < 0.001**

Fixation Indices:

FIS : 0.83410
FSC : 0.56443
FCT : 0.20845
FIT : 0.94280

Table 7: Pairwise Population Fst Values. (Fst Values are shown below diagonal. Probability values based on 1000 permutations are shown above diagonal). *APN* – Airport N, *APW* – Airport W, *APS* – Airport S, *APSR* – Airport S Runway, *BR* – Bidwell Ranch, *CH* - Church, *DM* – Doe Mill, *DR* – Hwy 149 N, *DRCBn* – Dove Ridge N, *DRCBw* – Dove Ridge W, *DRCBsw* – Dove Ridge SW, *DRCBe* – Dove Ridge E, *DRCBse* – Dove Ridge SE, *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	DR	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
DR	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

Appendix A: Extant BCM Collection Sites:

I obtained leaf tissue samples from a total of 21 sites (Table 1), and some of my collections overlap with those done by Dole & Sun (1992) and Dole (unpublished data).

I was able to survey three populations not previously sampled by Dole & Sun (1992) and Dole (unpublished report):

- **Table Mountain:** located along Cherokee Road within the North Table Mountain Ecological Reserve (http://www.dfg.ca.gov/lands/mgmtplans/ntmer/).
- **Wurlitzer Mitigation Site:** Created mitigation site north of Chico to mitigate impacts to the population north of the Doe Mill population (now residential area).
- **Hwy 149 North:** Owned by the Butte County Association of Governments and monitored by Restoration Resources, this site is adjacent (due south) to the Gallic-Evans Firing Range along Opens haw Road.

I was unable to collect from the following specific locations, previously surveyed by Dole & Sun (1992) and Dole (unpublished report), or reported by Clif Sellers (Appendix 1):

- **Airport Northeast** located on private property east of Cohasset Road, and **Diesel Lane** population, located east of Cohasset Road at the end of Diesel Lane, were both contiguous to the Bechtel Ranch population (Clif Sellers 2008 – Appendix 1). The Bechtel Ranch population is now part of the **Stone Ridge Ecological Reserve**, and was included in my 2008 genetic sampling.
- **West Rancho Arroyo** located adjacent to the 2008 sampled **Bidwell Ranch** property, a large piece of land that contains a very large extant BCM population. Whether the West Rancho Arroyo BCM population described by Clif Sellers (Appendix 1) still exists is not clear, as I was not able to access it in 2008.
- **Shippee Road** located approximately 17 km south of Chico along Shippee Road. I drove along the whole length of Shippee Road but could not locate this population, unless it is synonymous with populations within the **Dove Ridge Conservation Bank** which I sampled extensively.
- **Type population & 229A** located approximately 18 km south of Chico at the east side of Hwy 99 and Shippee Road. I surveyed within the **Dove Ridge Conservation Bank**, and the westernmost population is close to these sites. Whether it is the same or the closest remnant of the Type or 229A population is unclear. The southeast corner of Hwy 99 and Shippee Rd is now in agricultural use.
- **Stilson:** I did not get permission to go on this site and so was unable to access the entire population, but I sampled along the north & south sides of Stilson-Canyon road.

Appendix B:

Status of Populations of
Butte County Meadowfoam (*Limnanthes floccosa* ssp. *californica*)

Clif Sellers, ACSD
City of Chico
8 January 2008

Dole Populations

1. North Enloe/Church/Humboldt Population. Portions of this population located on both sides of Humboldt Road and west of Bruce Road have been both impacted and preserved. The entire population on the south side of Humboldt Road is within a 35 acre preserve which includes the entire watershed and buffers, and is protected by a conservation easement. Fee ownership remains with Enloe Hospital Foundation and the conservation easement is held by the City of Chico. An additional .5 acres of buffer was provided along the westerly edge with recent construction of the adjacent school. A portion of the population on the north side of Humboldt Road (Pleasant Valley Assembly of God) was eliminated through grading of approximately 14 acres of a 19 acre parcel undertaken pursuant to a permit issued by the Army Corps of Engineers. (No City permits were required or issued for this grading activity.) BCM on the westerly 5 acres of the 19 acre parcel and on the 10.8 acre parcel to the north were not directly affected by this grading. BCM on this remaining portion and adjacent parcel continue to be observed, but size, number and extent of these populations have not been quantified.

2. Bruce-Stilson Canyon Population. Currently, there is no change in the setting for this population. However, the owner of the property (Drake) has made application for a permit to fill wetlands on the site and is currently in consultation with the federal agencies. Initial response from the agencies is that the direct and indirect impacts to BCM must be significantly reduced from what is currently depicted in the application.

3. Doe Mill Population. The portion of this population north of Warfield Lane and Doe Mill Road west of the diversion channel was eliminated under permits issued by the Army Corps of Engineers and City approvals, with significant mitigation required (See Wurlitzer Ranch [#18] below). Based on more recent surveys, this population appears to be more extensive south of Warfield Lane and Doe Mill Road, on both sides of the diversion channel, than originally mapped by Dole. In spring 2002, several additional isolated populations were identified on the north side of Warfield Lane/Doe Mill Road east of the diversion channel, with the largest of the populations totaling approximately 50 plants and the cumulative total less than 150 plants. This population was eliminated by development of the Belvedere Subdivision in Spring 2006, with mitigation included under the permit discussed above. The most northerly portion of the population east of the diversion channel and south of Warfield Lane is contained within a 14.75 acre City owned preserve, managed for BCM preservation. For the remainder of this population, there is currently no change in the setting. However, the owner of the property containing the remainder of the Doe Mill Population (and the

Schmidbauer Population [#4] described below), Bruce Road Associates, has made application for a permit to fill wetlands on the site and is currently in consultation with the federal agencies. The application proposes preservation of all of the population east of the diversion channel and the majority of the population on the west side of the channel, and elimination of the Schmidbauer Population. Initial response from agencies is that the direct and indirect impacts to BCM must be reduced from levels currently depicted in the application.

4. Schmidbauer Population. There is currently no change in the setting. However, the owner of the property containing this population (and the remaining portions of the Doe Mill Population [#3] described above), Bruce Road Associates, has made application for a permit to fill wetlands on the site and is currently in consultation with the federal agencies. The application proposes elimination of this population and habitat, but includes preservation of major portions of the Doe Mill Population. Initial response from the agencies is that the direct and indirect impacts to BCM must be reduced from what is currently depicted in the application.

5. West Rancho Arroyo Population. This entire population is on a City owned 292 acre permanent preservation site (Foothill East Preserve). This preservation site includes wetlands, wetland mitigation and BCM, and is protected by a conservation easement. The population mapped by Dole is located along a fence line on the eastern boundary of the parcel and occupies only a small portion of the suitable habitat identified. With limitations on grazing now in place, this population may expand into additional habitat within the preserve, and in fact is now commonly found in shallow swales extending approximately 250 feet westerly from the fence line. This population is also contiguous to the BCM population on the City owned Bidwell Ranch site (see #17 below). (Apparently the late 1990s field work which formed the basis for establishing the 292 acre preserve, including wetland creation as mitigation, did not identify any BCM populations at this location. However, as this portion of the preserve was an avoidance area and "upstream" from the mitigation activities, the field work may not have been as thorough as otherwise expected. In addition, even if this population is not currently present, the opportunity for reestablishment exists with the presence of the "upstream" population on the City owned Bidwell Ranch site.)

6. North Airport Population. Subsequent surveys, including additional work by Dole, have found this population to be much smaller than estimated by Dole in 1988. This population is located in atypical habitat (fire break and/or wheel ruts), both in terms of hydrology and associated plant species, and subsequent surveys may reflect decline in the population resulting from habitat or other stress. The City of Chico has proposed an airport expansion which would eliminate a majority of this population. The City is proposing a mitigation program of avoidance in other areas of the airport, management for long term viability and restoration of BCM habitat at the airport. The City will also consider acquisition of additional adjacent lands with existing BCM populations for BCM preservation and management. The remaining portions of the population were isolated and located in atypical habitat, with long term existence questionable.

7. Airport West Population. This population has shown wide variations in size and range during the several surveys conducted. Additional habitat and plants may be

located on contiguous unsurveyed properties. The City's proposed airport expansion entirely avoids this population, although there may be indirect impacts. The City's proposal includes managing this area for resource conservation.

8. Airport South Population. This population is much more extensive than mapped by Dole in 1988. The City's proposed airport expansion entirely avoids the portion of this population on City owned lands, although there may be indirect impacts. The City will propose management of this area for BCM preservation as part of the airport master plan project. During wetland delineation on the private properties to the west of the Airport South population, additional occurrences of BCM and suitable habitat were noted, but a formal BCM survey was not conducted. A new north-south airport access road, proposed as part of the master plan, may affect portions of this BCM population dependent on how far the plant extends westerly on the adjacent properties. There were no other current proposals which would affect the portions of the population on the adjacent privately owned lands. The City may consider acquisition of portions of these properties as mitigation for impacts to the North Airport Population.

9. Airport Northeast Population. This small population, located on private property east of Cohasset Road, is not threatened by any development activity, but the area does continue to be heavily grazed. It is also contiguous to the Bechtel Ranch population (#16) described below.

Other Populations

10. Bruce Road Population. This population is located on the west side of Bruce Road north of State Highway Route 32 and was reported to the Natural Diversity Data Base in 1991. The site is in an area of ongoing urban development, and development of multiple family residential housing and commercial uses is being explored by the owners.

11. Shippee Road Population. This population is located approximately 17 km south of Chico along Shippee Road between Highways 99 and 149, and is reported as degraded due to agricultural and off road vehicle activities. There were no known changes in the condition of this population or habitat.

12. Type Population. This population is located approximately 18 km south of Chico on the east side of Highway 99, at the Shippee Road intersection. This population has been reported as eliminated due to conversion to agricultural use.

13. Highway 149 North Population. This significant population is south of Chico and about 3 km east of the Shippee Road Population. A major portion of the population is located on the publicly owned Gillick-Evans Firing Range (but limited to law enforcement agency use only) and protected within a 7 seven acre conservation easement with management and monitoring requirements. The adjacent properties were physically similar and probably contain additional portions of the population, but were not specifically surveyed as part of the firing range preservation work. CalTrans has completed an endangered species survey for portions of the population, but the survey is not available to the City. This population is north of the Highway 149 South Population. Aside from the firing range, lands in this area were used for seasonal grazing and not proposed for development.

14. Highway 149 South Population. This population is south of Chico and about 3 km east of the Shippee Road Population. Recent information places this population extending as far east as the intersection with State Highway Route 70, conflicting with the proposed Highway 149 widening (from news descriptions of the site in which the plant is found, it appears that it is not in typical BCM habitat). CalTrans has completed an endangered species survey for at least a portion of this population, but the survey is not available to the City. This population is south of the Highway 149 North Population. Lands in this area were used for seasonal grazing and not proposed for development. This land is now part of the Dove Ridge Conservation Bank.

15. Diesel Lane Population. This minor population is located east of Cohasset Road at the end of Diesel Lane. The status of this population is unknown, but it is likely that it has been adversely affected, if not entirely eliminated, by adjacent development activities approved by Butte County. Based on proximity to the Bechtel Population (#16), it is possible that these two populations represent plants in the same range of habitat.

16. Bechtel Ranch Population. This major population (150,000+ plants on approximately 135 acres of habitat) is located directly east of the airport across Cohasset Road and contiguous to Dole's Airport Northeast Population (#9). Jones & Stokes Associates conducted the BCM (and other listed species) survey in 1994. The Wildlife Conservation Board acquired the portion of the Bechtel Ranch containing the BCM population in late 2005/early 2006, as well as other contiguous areas with a variety of habitats.

17. Bidwell Ranch Population. The City owns the 750 acre Bidwell Ranch (formerly known as Rancho Arroyo) property, including 120+ acres of BCM habitat and buffers. Although no formal City action has been taken for implementation, it is generally acknowledged that the 120+ acres will be set aside for BCM conservation. The 120+ acres includes the entire BCM habitat on this property, the entire contributing watershed and 200 foot buffers. A protocol level survey was conducted by Gallaway Consulting, Inc. in April 2006, with the population limited to the most northerly drainage and containing almost 162,000 plants. This population is contiguous to the West Rancho Arroyo Population (#5) described above. The City has contracted with River Partners to establish a mitigation bank on the property.

18. Wurlitzer Ranch Preserve Population. This preserve was created to mitigate impacts to portions of the Doe Mill Population (see above), and included wetlands creation and BCM introduction in 1992. BCM was initially seeded on the preserve in 1992 and other portions of the site were seeded the following year. According to the preserve manager, David Kelley, and the annual monitoring reports, there has been no further BCM seeding since 1993. Based on seven years of monitoring, the BCM populations on the site appear to be stable or increasing in number. The 2000 survey work estimated BCM population at 200,000 plants, although the monitoring reports identify a population ranging from 11,000 to 22,000 plants for the areas used to demonstrate mitigation compliance. A conservation easement is in place. (Note: In 2007, Jody Gallaway, Gallaway Consulting Inc., has reported that this preserve/creation site is in serious decline

with low wetlands function, declining plant diversity, and dwindling BCM populations. This information has not been independently corroborated, but is worth noting for future evaluation. GCI is also working to establish a second Wurlitzer Preserve on contiguous properties.)

Appendix C: Allelic frequencies per population (allelic frequencies of 1.00 indicate that the population is fixed for the specified allele at the given locus).

Locus	LS527					LS02						
Allele/n	N	283	285	287	301	N	177	179	181	183	185	197
Airport N	12	0.00	1.00	0.00	0.00	10	0.00	1.00	0.00	0.00	0.00	0.00
Airport S	9	0.00	0.00	1.00	0.00	10	0.00	1.00	0.00	0.00	0.00	0.00
Airport S Runway	9	0.00	1.00	0.00	0.00	4	0.00	0.50	0.50	0.00	0.00	0.00
Airport W	10	0.00	1.00	0.00	0.00	10	0.00	1.00	0.00	0.00	0.00	0.00
Bidwell Ranch	28	0.00	1.00	0.00	0.00	27	0.00	1.00	0.00	0.00	0.00	0.00
Church	13	0.23	0.62	0.00	0.15	23	0.00	0.26	0.54	0.20	0.00	0.00
Doe Mill	20	0.00	1.00	0.00	0.00	16	0.00	1.00	0.00	0.00	0.00	0.00
Hwy 149 North	33	0.00	0.32	0.00	0.68	32	0.00	0.25	0.03	0.72	0.00	0.00
Dove Ridge E	19	0.00	0.53	0.00	0.47	21	0.00	0.00	1.00	0.00	0.00	0.00
Dove Ridge N	29	0.00	0.00	0.00	1.00	29	0.00	0.00	0.00	1.00	0.00	0.00
Dove Ridge SE	28	0.00	0.11	0.00	0.89	24	0.00	0.73	0.00	0.02	0.25	0.00
Dove Ridge SW	19	0.00	0.00	0.00	1.00	23	0.00	0.00	1.00	0.00	0.00	0.00
Dove Ridge W	13	0.00	0.00	0.00	1.00	14	0.00	0.14	0.86	0.00	0.00	0.00
North Enloe	35	0.00	1.00	0.00	0.00	36	0.00	0.31	0.67	0.01	0.00	0.01
Schmidbauer E	25	0.04	0.92	0.02	0.02	21	0.07	0.93	0.00	0.00	0.00	0.00
Schmidbauer SE	13	0.08	0.81	0.12	0.00	14	0.00	0.93	0.07	0.00	0.00	0.00
Schmidbauer W	22	0.23	0.77	0.00	0.00	22	0.00	0.91	0.07	0.00	0.00	0.02
Stone Ridge	24	0.00	1.00	0.00	0.00	14	0.00	0.93	0.07	0.00	0.00	0.00
Stilson Canyon	15	0.07	0.93	0.00	0.00	15	0.00	0.97	0.03	0.00	0.00	0.00
Table Mountain	24	0.56	0.44	0.00	0.00	25	0.00	0.92	0.08	0.00	0.00	0.00
Wurlitzer	26	0.12	0.88	0.00	0.00	24	0.00	0.25	0.75	0.00	0.00	0.00

Locus	LS166				LS43						
	N	229	232	244	N	221	230	232	234	238	240
Airport N	11	1.00	0.00	0.00	6	0.00	0.00	0.00	1.00	0.00	0.00
Airport S	9	1.00	0.00	0.00	10	0.00	0.00	0.00	1.00	0.00	0.00
Airport S Runway	9	1.00	0.00	0.00	9	0.00	0.00	0.00	1.00	0.00	0.00
Airport W	10	1.00	0.00	0.00	10	0.00	0.00	0.00	1.00	0.00	0.00
Bidwell Ranch	26	1.00	0.00	0.00	29	0.00	0.00	0.00	1.00	0.00	0.00
Church	21	1.00	0.00	0.00	22	0.00	0.02	0.30	0.68	0.00	0.00
Doe Mill	21	0.95	0.05	0.00	12	0.00	0.00	0.00	0.88	0.04	0.08
Hwy 149 North	34	0.96	0.03	0.01	27	0.00	0.00	0.15	0.74	0.07	0.04
Dove Ridge E	20	1.00	0.00	0.00	14	0.00	0.00	0.00	0.86	0.14	0.00
Dove Ridge N	28	1.00	0.00	0.00	11	0.09	0.00	0.00	0.82	0.09	0.00
Dove Ridge SE	29	1.00	0.00	0.00	20	0.00	0.00	0.00	0.70	0.30	0.00
Dove Ridge SW	22	1.00	0.00	0.00	14	0.00	0.00	0.00	0.89	0.11	0.00
Dove Ridge W	12	1.00	0.00	0.00	2	0.00	0.00	0.00	0.50	0.50	0.00
North Enloe	36	1.00	0.00	0.00	36	0.00	0.00	0.19	0.79	0.01	0.00
Schmidbauer E	25	1.00	0.00	0.00	21	0.00	0.00	0.00	0.88	0.12	0.00
Schmidbauer SE	14	1.00	0.00	0.00	14	0.00	0.00	0.00	1.00	0.00	0.00
Schmidbauer W	23	1.00	0.00	0.00	23	0.00	0.00	0.00	1.00	0.00	0.00
Stone Ridge	24	0.31	0.00	0.69	24	0.00	0.00	0.00	1.00	0.00	0.00
Stilson Canyon	15	1.00	0.00	0.00	15	0.00	0.00	0.00	1.00	0.00	0.00
Table Mountain	23	0.33	0.67	0.00	24	0.00	0.00	0.00	0.19	0.81	0.00
Wurlitzer	27	1.00	0.00	0.00	25	0.00	0.00	0.00	1.00	0.00	0.00

Locus	LS122				LS184						
	N	391	397	399	N	313	315	317	319	321	325
Airport N	12	1.00	0.00	0.00	12	0.00	0.00	0.00	1.00	0.00	0.00
Airport S	10	1.00	0.00	0.00	9	0.00	0.00	0.00	1.00	0.00	0.00
Airport S Runway	9	1.00	0.00	0.00	9	0.00	0.00	0.00	0.89	0.11	0.00
Airport W	10	1.00	0.00	0.00	10	0.00	0.00	0.00	0.00	1.00	0.00
Bidwell Ranch	29	1.00	0.00	0.00	29	0.00	0.00	0.79	0.17	0.03	0.00
Church	23	0.98	0.00	0.02	21	0.00	0.00	0.98	0.02	0.00	0.00
Doe Mill	20	1.00	0.00	0.00	21	0.00	0.14	0.69	0.00	0.17	0.00
Hwy 149 North	35	1.00	0.00	0.00	34	0.00	0.51	0.00	0.09	0.38	0.01
Dove Ridge E	19	1.00	0.00	0.00	15	0.00	0.00	0.00	1.00	0.00	0.00
Dove Ridge N	28	1.00	0.00	0.00	29	0.00	0.00	0.00	1.00	0.00	0.00
Dove Ridge SE	27	1.00	0.00	0.00	29	0.03	0.14	0.83	0.00	0.00	0.00
Dove Ridge SW	17	1.00	0.00	0.00	20	0.00	0.00	0.00	0.00	1.00	0.00
Dove Ridge W	14	1.00	0.00	0.00	14	0.00	0.00	0.00	0.96	0.04	0.00
North Enloe	33	1.00	0.00	0.00	28	0.00	0.00	0.46	0.18	0.36	0.00
Schmidbauer E	24	1.00	0.00	0.00	24	0.21	0.67	0.00	0.00	0.13	0.00
Schmidbauer SE	13	1.00	0.00	0.00	14	0.00	0.00	0.43	0.00	0.57	0.00
Schmidbauer W	22	1.00	0.00	0.00	23	0.04	0.09	0.87	0.00	0.00	0.00
Stone Ridge	22	1.00	0.00	0.00	24	0.00	0.77	0.00	0.23	0.00	0.00
Stilson Canyon	15	1.00	0.00	0.00	15	0.00	0.00	0.23	0.00	0.77	0.00
Table Mountain	23	0.50	0.50	0.00	22	0.00	0.00	1.00	0.00	0.00	0.00
Wurlitzer	27	1.00	0.00	0.00	28	0.00	0.07	0.04	0.00	0.89	0.00

Locus	LS164							LS179					
	N	237	249	251	253	255	257	N	278	280	282	284	286
Airport N	12	0.00	0.17	0.83	0.00	0.00	0.00	12	0.00	0.00	0.50	0.42	0.08
Airport S	10	0.00	0.00	0.00	1.00	0.00	0.00	8	0.00	0.00	0.50	0.00	0.50
Airport S Runway	9	0.00	0.00	0.00	1.00	0.00	0.00	9	0.00	0.00	0.39	0.00	0.61
Airport W	10	0.00	0.00	1.00	0.00	0.00	0.00	9	0.00	0.00	0.50	0.50	0.00
Bidwell Ranch	29	0.00	0.00	0.62	0.38	0.00	0.00	29	0.00	0.00	0.72	0.28	0.00
Church	19	0.00	0.00	0.00	0.89	0.00	0.11	19	0.00	0.00	0.53	0.13	0.34
Doe Mill	21	0.00	0.05	0.76	0.19	0.00	0.00	19	0.00	0.00	0.68	0.08	0.24
Hwy 149 North	35	0.29	0.09	0.00	0.00	0.63	0.00	31	0.00	0.00	0.63	0.34	0.03
Dove Ridge E	20	0.00	0.00	0.20	0.80	0.00	0.00	7	0.00	0.00	0.07	0.57	0.36
Dove Ridge N	29	0.00	1.00	0.00	0.00	0.00	0.00	27	0.04	0.11	0.52	0.33	0.00
Dove Ridge SE	28	0.00	0.00	0.00	0.11	0.36	0.54	12	0.00	0.00	0.50	0.50	0.00
Dove Ridge SW	19	0.00	0.00	0.00	0.03	0.08	0.89	20	0.00	0.00	0.53	0.48	0.00
Dove Ridge W	14	0.00	0.00	0.00	0.00	1.00	0.00	12	0.08	0.08	0.42	0.42	0.00
North Enloe	36	0.00	0.00	0.07	0.74	0.19	0.00	37	0.00	0.00	0.59	0.35	0.05
Schmidbauer E	25	0.00	0.00	0.94	0.06	0.00	0.00	25	0.00	0.00	0.82	0.16	0.02
Schmidbauer SE	14	0.00	0.00	0.89	0.11	0.00	0.00	13	0.00	0.00	0.46	0.04	0.50
Schmidbauer W	23	0.00	0.00	0.83	0.17	0.00	0.00	11	0.00	0.00	0.77	0.23	0.00
Stone Ridge	24	0.00	0.54	0.46	0.00	0.00	0.00	23	0.00	0.00	0.52	0.00	0.48
Stilson Canyon	15	0.00	0.00	0.00	0.87	0.13	0.00	15	0.00	0.00	0.67	0.00	0.33
Table Mountain	23	0.00	0.00	0.83	0.17	0.00	0.00	17	0.00	0.00	0.85	0.09	0.06
Wurlitzer	27	0.00	0.07	0.04	0.07	0.81	0.00	29	0.00	0.02	0.53	0.41	0.03

Locus	LS321								
	N	271	273	275	277	279	281	283	287
Airport N	9	0.00	0.00	0.00	0.00	0.11	0.78	0.11	0.00
Airport S	8	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Airport S Runway	4	0.00	0.00	0.00	0.25	0.75	0.00	0.00	0.00
Airport W	10	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Bidwell Ranch	23	0.00	0.00	0.00	0.17	0.83	0.00	0.00	0.00
Church	21	0.00	0.00	0.00	0.10	0.74	0.17	0.00	0.00
Doe Mill	19	0.00	0.00	0.00	0.00	0.95	0.05	0.00	0.00
Hwy 149 North	35	0.00	0.06	0.00	0.74	0.10	0.09	0.00	0.01
Dove Ridge E	12	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Dove Ridge N	29	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Dove Ridge SE	29	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Dove Ridge SW	17	0.06	0.59	0.00	0.06	0.29	0.00	0.00	0.00
Dove Ridge W	14	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
North Enloe	23	0.00	0.00	0.00	0.04	0.87	0.09	0.00	0.00
Schmidbauer E	25	0.00	0.00	0.00	0.04	0.44	0.52	0.00	0.00
Schmidbauer SE	14	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Schmidbauer W	20	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Stone Ridge	21	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Stilson Canyon	14	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Table Mountain	25	0.00	0.00	0.02	0.04	0.94	0.00	0.00	0.00
Wurlitzer	28	0.00	0.00	0.00	0.86	0.04	0.11	0.00	0.00

Appendix D: Hardy Weinberg Equilibrium

	APN	APS	APSR	APW	BR	CH	DM	Hwy149N	DRCBe	DRCBn	DRCBse	DRCBsw	DRCBw	NE	SCHe	SCHse	SCHw	SR	STN	TM	WU
LS527	m	m	m	m	m	0.000***	m	0.000***	0.000***	m	0.000***	m	m	m	0.000***	0.000***	0.000***	m	0.000***	0.000***	0.000***
LS02	m	m	0.046*	m	m	0.000***	m	0.000***	m	m	0.000***	m	0.000***	0.000***	0.003**	0.000***	0.000***	0.000***	0.894	0.000***	0.000***
LS166	m	m	m	m	m	m	0.000***	0.000***	m	m	m	m	m	0.000***	m	m	m	0.533	m	0.015*	m
LS43	m	m	m	m	m	0.000***	0.007**	0.000***	0.119	0.000***	0.001***	0.019*	0.157	0.000***	0.144	m	m	m	m	0.258	m
LS122	m	m	m	m	m	0.915	m	m	m	m	m	m	m	m	m	m	m	m	m	0.000***	m
LS179	0.007**	0.005**	0.056	0.003**	0.040*	0.002**	0.047*	0.000***	0.063	0.000***	0.021*	0.000***	0.000***	0.191	0.006**	0.671	0.329	0.006**	0.053	0.918	0.001**
LS164	0.001***	m	m	m	0.000***	0.000***	0.000***	0.000***	0.000***	m	0.000***	0.000***	m	0.000***	0.001**	0.019*	0.000***	0.000***	0.000***	0.000***	0.000***
LS184	m	m	0.003**	m	0.000***	0.911	0.000***	0.000***	m	m	0.000***	m	0.890	0.000***	0.000***	0.000***	0.000***	0.000***	0.002**	m	0.000***
LS321	0.000***	m	0.046*	m	0.000***	0.000***	0.000***	0.000***	m	m	m	0.001***	m	0.000***	0.000***	m	m	m	m	0.000***	0.000***
m - monomorphic, * P<0.05, ** P<0.01, *** P<0.00																					

APENDIX E: Number of linked loci per locus, P< 0.05.

	APN	APS	APSR	APW	BR	CH	DM	Hwy149N	DRCBe	DRCBn	DRCBse	DRCBsw	DRCBw	NE	SCHe	SCHse	SCHw	SR	STN	TM	WU
LS527	0	0	0	0	0	5	0	4	2	0	2	0	0	0	2	2	1	0	0	1	4
LS02	0	0	1	0	0	2	0	4	0	0	2	0	0	3	0	0	1	1	0	0	4
LS166	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	2	0	2	0
LS43	0	0	0	0	0	4	2	3	1	0	2	1	0	1	2	0	0	0	0	1	0
LS122	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
LS179	1	0	0	0	1	1	1	0	3	0	1	0	0	2	1	0	2	1	0	2	3
LS164	2	0	0	0	2	2	2	3	2	0	2	0	0	2	1	1	0	1	1	4	5
LS184	0	0	0	0	1	1	3	4	0	0	1	0	0	4	2	1	2	1	1	0	5
LS321	1	0	1	0	2	2	0	3	0	0	0	1	0	4	2	0	0	0	0	1	5

APPENDIX F: Effective Population Size per Occurrence, P< 0.05.

	APN	APS	APSR	APW	BR	CH	DM	HWY149N	DRCBe	DRCBn	DRCBse	DRCBsw	DRCBw	NE	SCHe	SCHse	SCHw	SR	STN	TM	WU
Harmonic Mean	9.6	0	4.6	0	26	15.4	15.2	29.9	7.9	11	17.6	14	12	28.2	22.9	12.9	17.2	18.3	15	19.5	26
R^2	0.5	0	0.42	0	0.28	0.16	0.09	0.04	0.21	0.09	0.09	0.03	0.05	0.06	0.11	0.17	0.06	0.14	0.14	0.04	0.4
Estimated Ne	0.4	0	14.9	0	0.7	1.9	35.1	63.7	9.7	-12.1	12.4	-6.4	-6.4	12.9	2.8	2	-28.1	2	2.9	-15.3	0.4

APPENDIX G: SSR Marker GeneBank Accession Numbers

SSR Marker	GenBank Accession Number
LS527	BV007349
LS02	BV007038
LS166	BV007132
LS43	BV007051
LS122	BV007107
LS179	BV007141
LS164	BV007130
LS184	BV007142
LS321	BV007217