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Evolutionary potential of a dispersal-restricted species in response to climate change

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EVOLUTIONARY POTENTIAL OF A DISPERSAL-RESTRICTED SPECIES IN RESPONSE TO CLIMATE CHANGE

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To my Dad, Mom and Sister who gave me the strength to get here.

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ABSTRACT

Torres Martinez, Lorena. Ph.D., Purdue University, August 2016. Evolutionary Potential of a Dispersal-Restricted Species in Response to Climate Change. Major Professors: Morris Levy and Nancy C. Emery.

Habitat replacement and fragmentation associated with projected climate change pose a critical threat to global biodiversity. Edaphically limited plant species with restricted dispersal abilities will be especially handicapped to track their optimal climate spatially. Instead, the persistence of these species will depend on their capacity to adapt *in situ* to novel climate regimes. Here I evaluated the evolutionary potential of *Lasthenia fremontii,* an annual plant species restricted to ephemeral wetlands called vernal pools in California to adapt to the projected patterns of climate change. Across *L. fremontii* distribution there is a latitudinal gradient in precipitation which, combined with reduced gene flow rates, might be driving adaptive divergence in climate tolerances among populations of this species. Accordingly, I estimated (1) the spatial distribution of genetic variation and gene flow across the species range, (2) the extent to which the climate variability experienced by the vernal pools has selected for seed dormancy in *L. fremontii* populations, and (3) the degree of local adaptation and additive genetic variation in response to a simulated spectrum of precipitation conditions. My analyses revealed an isolation-by-distance model of genetic differentiation among vernal pools and

a low to moderate degree of genetic differentiation among pools within a single complex. Germination time was faster in the northernmost (historically wettest) population than in the southernmost (historically driest) population but with mixed responses in others. I observed a significant positive relationship between the historical variability in autumn precipitation and extent of seed dormancy in a population. These findings were consistent with the patterns of adaptation to local rainfall conditions observed among three of the populations reciprocally exposed to local but extreme precipitation conditions. Unexpectedly, however, populations expressed higher levels of additive genetic variation but reduced fitness under extreme drought events in comparison with moderate and extreme rainfall conditions. Further, both peripheral populations expressed optimal fitness in their native conditions but the central population did not. Taken together, these results revealed that restricted gene flow, coupled with differences in the history of local selection pressures, have led to significant divergence in the climatic tolerances and relative evolutionary potential of populations. Contrary to intuitive expectations, central range populations with less predictable climate regimes may not preserve adaptive potential for more extreme environments. That potential may only be present at the current environmental extremes.

CHAPTER 1. GENOME-WIDE SNP DISCOVERY IN THE ANNUAL HERB, *LASTHENIA FREMONTII* (ASTERACEAE): GENETIC RESOURCES FOR THE CONSERVATION AND RESTORATION OF A CALIFORNIA VERNAL POOL ENDEMIC

1.1 Introduction

Reduced-genome representation methods can significantly increase the availability of molecular resources for the conservation of non-model species by providing relatively quick and cost-effective tools for developing molecular markers (Hohenlohe et al. 2011, Catchen et al. 2013). Restriction-site-associated DNA sequencing (RADseq) circumvents the complexity and expense of whole-genome sequencing by sequencing a random subsample of the genome. This technique allows the discovery of thousands of single nucleotide polymorphisms (SNPs) (Miller et al. 2007, Baird et al. 2008, Davey and Blaxter 2011), and thus is becoming a widespread method for generating sequence data that can in turn be used to evaluate phylogeographic (e.g, Emerson et al. 2010) and phylogenetic patterns (e.g., Eaton and Ree 2013), conduct genome-wide association and QTL analyses (e.g., Baxter et al. 2011, Wu et al. 2014), estimate demographic and population structure patterns (e.g., Hohenhole et al. 2010, Corander et al. 2013), identify introgressive gene flow following divergence events (e.g., Eaton and Ree 2013, Catchen et al. 2013a), and identify regions of the genome that are

under selection and involved in local adaptation (e.g. Hecht et al. 2015, Gaither et al. 2015). RADseq can potentially discover thousands of molecular markers in a species' genome using a small number of individuals, providing yet another advantage for studies that target rare and endangered species for which sample sizes can be severely limited (Narum et al. 2013).

I used RADseq to develop SNP markers for an indicator species of vernal pool ephemeral wetlands of the California Floristic Province, a heavily threated habitat type that supports a diverse community of endemic fauna and flora. Vernal pools in California (USA) are heavily threatened by extensive habitat loss due to urban and agricultural expansion (Griggs and Jain 1983) and high projected rates of climate change in the region (Loarie et al. 2009, Halbur et al. 2014). These geographically isolated, ephemeral wetlands are typically clustered into groups called "vernal pool complexes" that are thought to function as "island archipelagoes" in a grassland "ocean" (Holland and Jain 1981, Zedler 2003). Pools develop in regions where naturally-occurring depressions in the grasslands overlay a restricting soil horizon that prevents the downward drainage of water during the cool, wet winters that are characteristic of the mediterranean climate in this region (Keeler-Wolf et al.1998, Zedler 2003). The pools rapidly dry each spring as precipitation declines and temperatures rise, and remain dry during the summer season (Holland and Jain 1981, Zedler 2003). A large number of endemic plant species that have adapted to tolerate the annual cycles of flooding and drought are restricted to, or highly associated with, VP habitat in the California Floristic Province (Stone 1990).

Lasthenia fremontii (Madieae, Asteraceae), commonly known as Fremont's Goldfields, is a widespread and locally abundant vernal pool plant species that is endemic

to the vernal pools of California (Barbour et al. 2007, Emery 2009), and is considered an indicator species for the habitat and specific VP community types (Keeler-Wolf et al.1998, Barbour et al. 2005, Barbour et al. 2007). Additionally, *L. fremontii* is closely related to two federally endangered vernal pool species, *Lasthenia burkei* and *Lasthenia conjugens* (Chan 2001). All three species are annual, obligate-outcrossing herbs that may be dispersal-limited due to the island-like nature of VP habitat and the reduced pappus (hairs or bristles that facilitate wind dispersal) compared to its terrestrial relatives (Ornduff 1966). Gene movement by pollen may be limited due to the localized pollinator foraging behavior (Emery et al. 2011a). Collectively, these factors may promote genetic differentiation and local adaptation of populations within species that may influence the success of restoration and mitigation efforts that involve seed translocation (Elam 1998). To date, only one study has characterized local patterns of genetic diversity in *L. fremontii*. Using allozymes, Crawford and Ornduff (1989) detected some differentiation among *L. fremontii* populations (G_{st}=0.09) among five different localities that spanned three adjacent counties near the center of the species' range.

Compared to classical molecular tools (including allozymes), modern molecular markers like SNPs subsample whole-genome diversity and thus provide substantially more information for inferring population structure and dynamics (Morin et al. 2009). Additionally, SNPs follow simple mutation models of evolution, like the infinite sites model (Morin et al. 2004, Morin et al. 2009), which makes them suitable for estimating historical demographic events (e.g., bottlenecks and introgression). These properties, combined with the dispersion and abundance of SNPs throughout the genome, makes these molecular markers particularly useful for assessing genome-wide patterns of

genetic variation in rare and threatened populations. Therefore, developing a full set of SNP markers for *L. fremontii* will substantially enhance the potential for genetic analyses that can inform the conservation and management of vernal pool populations.

In this study, I first generated genomic RAD-tags to discover candidate SNP markers for *L. fremontii*. Then, I used a subset of these SNPs to evaluate the distribution of genetic variation in *L. fremontii* at two spatial scales: (1) among vernal pool complexes that collectively span the species' geographic range, and (2) among individual pools within VP complexes. Finally, I provide a preliminary analysis of the distribution of genetic variation in *L. fremontii* illustrate the utility of these genomic resources at each spatial scale.

1.2 Materials and Methods

1.2.1 Sampling design and DNA isolation

I collected seeds from 12 different vernal pool complexes in California, with balanced representation of the northern, central, and southern regions of the species' geographic range (Figure 1.1A, Table 1.1). Collections were performed in two complexes in 2008 and ten complexes in 2013 (Figure 1.1A, Table 1.1). At each complex, seeds were harvested from 15 – 20 different maternal plants in up to 4 different vernal pools (Table 1.1). To provide an in-depth analysis of genetic variation among subpopulations (i.e., different pools) within a VP complex, I sampled 10 individuals from each of 9 different pools at Mather Field in the spring of 2011 (Figure 2A). All collections took place during the spring season (April – May) so that species identity could be confirmed using floral and fruit characters. A random subset of the seeds from these collections was germinated and raised to maturity in a growth chamber at Purdue University. Genomic DNA was purified from leaf and stem tissues using Plant DNAeasy Kit (Qiagen Inc) with minor modifications to the manufacturer's instructions (i.e., increasing both lysis and DNA elution times) to increase yield. DNA concentration was assessed using fluorometry (Qubit -Invitrogen).

1.2.2 RAD-tag library preparation

I selected one plant from each of the 12 vernal pool complexes for SNP discovery (Figure 1A). The DNA from these 12 samples was pooled into a single RAD library using the approach described in Baird et al. (2008). This sampling design was used to provide a balanced representation (4 VP complexes each) of the northern, central, and southern portions of the species' range, and to maximize the potential SNP diversity that

could be discovered by including one representative from each different complex. I did not include multiple representatives from each VP complex to ensure that I had sufficient depth of coverage for each RAD-tag sequence, given a conservative estimate of genome size based on another Asteraceae, *Helianthus annus*, of 3.5 billion base pairs (Baack et al. 2005). To prepare the library, each DNA sample was diluted to 500 ng in 40 μl of PCR-grade water and digested with the restriction enzyme SbfI (NEB). I selected this enzyme due to its low cutting frequency, which is appropriate for reducing the complexity of large genomes to allow adequate depth of sequencing coverage for each RAD-tag. Furthermore, I wanted to maximize the discovery of nuclear SNPs, as opposed to chloroplast SNPs that are only maternally inherited, and an analysis of the chloroplast genome of *L. burkei* (Walker et al. 2014) indicated that there are no Sbf1 cutting sites for this restriction enzyme in its plastome.

After Sbf1 digestion, the fragments from each individual were ligated to an Illumina P1 adapter with a 6 bp barcode (Table A.1) that uniquely identified each sample (Miller et al. 2007). The twelve barcodes all differed by at least two nucleotides to minimize sample mis-assignment due to sequencing error. Adapter-ligated fragments were pooled and sheared for 6 minutes using a Sonic Ruptor 400 (OMNI, Inc.) to obtain an average fragment size of 500 bp. The samples were then purified with a MinElute PCR Purification Kit (Qiagen, Inc.) and subsequently separated by electrophoresis in a 2% agarose gel. I isolated fragments in the 300 – 600bp size range using a MinElute Gel Extraction Kit (Qiagen, Inc.). The dsDNA ends were treated with a blunting enzyme mix (NEB, Inc) at 20°C for 60 minutes to remove the overhangs, followed by the addition of 3’-adenine overhangs with 15U of Klenow 3 – 5’ exo-fragment (NEB, Inc), 1.0 μl of

dATPs and 5.0 µl of 10X NEBuffer2 (NEB, Inc), followed by an incubation at 37°C for 60 minutes. Next, the P2 adapter was ligated to each DNA fragment by adding 1 µl of 10 µM of the P2 adapter, using the method described above for the ligation of the P1 adapter. After re-purification of the sample, 10 µl were used as the template for a 100 µl PCR containing 50 µl of Phusion Master Mix (NEB), 2 µl of 10 µM P1 adapter primer (Illumina), 2 µl of 10 µM P2 adapter primer (Illumina) and 36 µl of water. The Phusion PCR cycling conditions were 98°C for 30 seconds, followed by 18 cycles of 98°C for 10 seconds, 65°C for 30 seconds, 72°C for 30 seconds, and a final extension of 72°C for 5 minutes. The final PCR product was purified and eluted with 20 µl of Elution Buffer (Qiagen). The entire product was separated in a 2% agarose gel, and the 500 bp target band was excised and purified with the MinElute Gel Extraction Kit (Qiagen). The concentration of the final library was quantified with qPCR. Finally, the library was submitted to the Genomics Center at Purdue University to be sequenced on one lane of Illumina Hi-Seq 2500 using singe-end rapid chemistry.

1.2.3 RAD-tag analysis and SNP discovery

The raw sequences of all 12 individuals were quality-filtered using Trimmomatic (Bolger et al. 2014) to remove adapters and discard reads with mean Phred quality scores < 20 (i.e., with a base call accuracy below 80%). The filtered reads were separated by barcode using the *process_radtags* pipeline in STACKS v.1.02 (Catchen et al. 2011, 2013). The pipeline also corrected and recovered reads with sequencing errors on either the barcode or restriction site (parameter "-r") while discarding reads with an average Phred score < 10 (90% correct) over 15% of the read length. The shearing step produces reads with different lengths. In my data set, most raw reads had a length ≥ 85bp, and I

observed an over-representation of SNPs after the 80 bp position (Table A.2), which is a common signal of terminal sequencing errors (Pujolar et al. 2013). To remove this source of SNP variation due to sequencing error, I further trimmed sequence ends to a final length of 75bp and discarded all reads < 75bp in length to obtain a final set of high-quality, equally-sized reads while minimizing the prevalence of false SNPs. I used the *denovo_map.pl* pipeline in STACKS v.1.02 (Catchen et al. 2011, 2013) to perform *de novo* locus assembly and SNP calling. This pipeline requires the user to specify the minimum stack depth required to establish an allele (parameter "-m"), the number of nucleotides allowed to differ among putative alleles that are assigned to the same locus within individuals (parameter "-M"), and the number of nucleotides allowed to differ among individuals and assigned to the same catalog locus (parameter "-n"). The values assigned to these parameters can lead to under- or over-merging of loci, and the optimal parameters values will vary among data sets. I systematically evaluated different combinations of these three parameters to ensure robust SNP calling among my samples (Catchen et al. 2013b, Mastretta-Yanes et al. 2014; Table A.2). Ultimately, the parameters that I considered both informative and conservative for my data set were: m=10, M=2, n=2 (catalog ID number 7 in Table A.2). I identified RAD-tag loci with extremely high coverage, which usually result from repetitive regions in the genome (Catchen et al. 2013b), using the –t option in STACKS, and then discarded these loci from subsequent analysis.

1.2.4 SNP selection and validation

To identify SNPs that were spread throughout the species' range and not bias my sample toward a few particularly divergent or polymorphic populations, I selected

polymorphic catalog loci that were present in at least 10 of the 12 individuals in the RAD library. My library design, which included one individual per population, prevented us from using a SNP selection procedure that involves ranking the SNPs by F_{ST} values and identifying those that best capture population structure (e.g., Hohenlohe et al. 2011). Next, I removed all catalog loci having > 3 SNPs and < 20 bp of flanking sequence on either side of the SNP. In catalog loci that contained multiple SNPs, I focused on the SNP with the longest flanking sequences, which facilitated primer design. Through this process, I generated a total of 100 candidate SNPs from the RAD library. Primers were successfully designed for 71 of the 100 SNPs (see *Chapter 1: Results*); these 71 SNPs were subsequently genotyped in 285 *L. fremontii* individuals spanning ten vernal pool complexes (Tables 2 and 3). SNP loci that were monomorphic or with a failure rate ≥ 30% were excluded for downstream analysis. Primer design, primer validation, and genotyping of the 285 individuals were performed by LGC genomics (Beverly, MA, USA) using a competitive allele-specific PCR (KASP) genotyping assay (Semagn et al. 2013).

1.2.5 Analysis of genetic variation

Forty-four (44) of the 71 SNPs were polymorphic among the 285 genotyped individuals and subsequently used to provide a preliminary analysis of the distribution of genetic variation among geographic regions and VP complexes. I tested for departures from Hardy-Weinberg equilibrium (HWE) at each locus within each VP complex using Arlequin version 3.5.1.2 (Excoffier and Lishner 2010). Using the polymorphic SNPs that were consistent with HWE, I estimated Weir and Cockerham's (1984) population pairwise F_{ST} values using the package *diveRsity* in R (Keenan et al. 2013). Bias-corrected

95% confidence intervals were calculated for each pairwise comparison between populations from 10,000 bootstrap re-samplings of individual genotypes within each population. Then, using the package *adegenet* (Jombart 2008; Jombart and Ahmed 2011), I performed a principal coordinate analysis (PCoA) using the pairwise F_{ST} comparisons as measures of the genetic distances between populations. A Euclidean matrix of the pairwise F_{ST} values was established using the *cailliez* option in *adegenet*. Additionally, I corroborated the pairwise F_{ST} comparisons and tested their statistical significance by permuting haplotypes between populations 10,000 times using Arlequin version 3.5.1.2 (Excoffier and Lishner 2010). I also estimated the degree of isolation-by-distance among vernal pool complexes using a Mantel test, which tested for a relationship between a matrix of linearized F_{ST} values (Slatkin 1995) and all pairwise distances between VP complexes (obtained from GPS coordinates). The statistical significance of the Mantel test was evaluated using a permutation test in GeneAlex version 6.5 with 9,999 permutations (Peakall and Smouse 2006, 2012).

I conducted a hierarchical analysis of molecular variance (AMOVA) using Arlequin version 3.5.1.2 (Excoffier and Lishner 2010) using the genotypic data to evaluate the distribution of genetic variation in *L. fremontii* at multiple spatial scales. In the large-scale analyses, the model partitioned genetic variation among geographic regions (north, central, or south), among VP complexes within regions, among individuals within VP complexes, and within individuals (see below for within-VP complex analysis). I also calculated the average number of alleles per locus across the 44 candidate SNPs using GeneAlex version 6.5 (Peakall and Smouse 2006, 2012).

I evaluated the fine-scale distribution of SNP variation among subpopulations within a single vernal pool complex using the genotype data that were obtained from the high-density sampling within Mather Field. This data set consisted of 91 genotypes from 9 different vernal pools (~10 individuals/pool) at Mather Field (see *Chapter 1: Materials & Method: Sampling design and DNA isolation*). Using the SNP data from these genotypes, I conducted the same analyses described above for the geographic analyses of populations (departures from HWE, $F_{ST,}$ isolation-by-distance, and Mantel tests) in which I treated each pool as a distinct subpopulation within the Mather Field VP complex.

1.3 Results

1.3.1 RAD-tag processing for SNP discovery and validation

Of the 80,726,565 high-quality reads that were obtained from the library, 32,477,626 were matched to the sample barcodes. The RAD library sequencing generated an average of 2.70 million reads/individual, with a range of 1.08 to 5.34 million reads (Tables 1.4 and A.1). A total of 32,084,041 reads (40% of the total reads obtained) were retained after trimming and removing reads with < 75bp length, leaving an average of 2.67 million reads per individual. From these reads a total of 316,106 catalog loci across all twelve individuals were obtained. A total of 754 (0.24%) catalog loci were present in ten or more individuals; of these, 713 (0.22%) were polymorphic, yielding 3918 candidate SNPs with an average of 5.8 SNPs per locus (range 1 – 22). Less than two-thirds of the 3918 candidate SNPs were transitions, with an observed transition to transversion ratio (Ti/Tv) of 1.7:1.

I filtered the 713 polymorphic loci for those that contained 3 or fewer SNPs (Table 1.4), which reduced the data set to 238 catalog loci. Of these, I selected 100 loci with large enough flanking sequences around the SNP to meet the criteria for primer design and testing by LGC Genomics. After sending these sequences to LGC genomics, 29 of the 100 loci considered to be not suitable for optimal primer design (Table A.3) and so could not be pursued further. Of the 71 putative SNPs that were suitable for optimal primer design, 9 (12.7%) failed to amplify in any individual sample, 4 (5.6%) failed for ≥30% of the samples, and 14 (19.7%) were monomorphic. As a result, 44 (62% of the 71 evaluated) SNPs were used in a preliminary analysis of the distribution of genetic variation in *L. fremontii* (Table A.4).

1.3.2 Analysis of genetic variation

Of the 285 *L. fremontii* individuals that were submitted for genotyping, 249 successfully amplified at ≥ 50% of the 44 diagnostic SNPs; 158 of these were from the range-wide collection (i.e., the Mather Field 2013 collection and the other nine vernal pool complexes), and the remaining 91 individuals were from the fine-scale collection at Mather Field in 2011. In the larger, range-wide collection of 158 individuals, an average of 14 individuals failed to amplify at a given diagnostic SNP (Table A.4).

My preliminary analyses of population genetic structure using the final data set of 44 SNP loci revealed genetic differentiation among *L. fremontii* populations from different VP complexes. The average number of loci with < 8% missing data across VP complexes was 29.4 ± 2.83 SE, and an average of 21 ± 0.80 loci were in HWE (Tables 1.2 and A.5). However, only an average of 15.4 ± 0.76 SE SNPs were polymorphic across VP complexes (Table 1.2) due to a relatively high number of fixed alleles per VP complex. The actual number of polymorphic loci characterizing an individual VP complex varied between 12 (CARR) and 20 (NTM) [Tables 1.2 and A.5]. Overall, a total of 33 SNPs both met the assumption of HWE and were not monomorphic across all VP complexes (Table A.5), and were thus used to estimate patterns of genetic diversity within and among VP complexes. At least three out of the ten complexes had private alleles with NTM presenting the highest number of unique alleles (3), followed by CARR (2), VINA (1) and JP (1). The average observed and expected heterozygosity values across VP complexes were 0.24 ± 0.01 SE and 0.28 ± 0.01 SE, respectively (Table 1.2).

The hierarchical analysis of molecular variance (AMOVA) and pairwise F_{ST} statistics also revealed significant genetic differentiation and population structure in

L. fremontii (Table 1.5). Approximately 12% of the total genetic variation in the data set was distributed among regions, 9% among VP complexes within regions, and 22% among VP complexes ($F_{ST} = 0.22$). Pairwise F_{ST} values among VP complexes were an average of 0.14 ± 0.01 SE and ranged from 0.00 – 0.31 (Table 1.6). The spatial distribution of the average F_{ST} value for each VP complex revealed a northeast to southwest gradient in population structure across the species' range, and particularly high levels of differentiation among populations from VP complexes at the southern range edge (Figure 1.1B). The most marginal *L. fremontii* populations were the most genetically distinct (i.e., exhibited the largest average F_{ST} values when compared to all other populations), e.g., the northwestern-most (DL and NTM) and the southeastern-most (PVP and CARR) VP complexes had mean F_{ST} values ≥ 0.20 (Table 1.5). The geographic gradient in genetic variation was also evident in the first coordinate of the PCoA, which explained 48% of the total variation in the data set (Figure 1.1B, x-axis). The populations from complexes in the northern and central portion of the species range were more genetically similar to each other than to the southern complexes with pairwise F_{ST} values ≤ 0.17. The second PCoA coordinate, which explained 17% of the total variation, was driven by the strong differentiation of the population from the DL complex relative to the populations in most other complexes, and especially BTM and PVP (Figure 1.1B, y-axis). In general, the observed patterns of genetic variation were statistically supported by an isolation-by-distance model ($R^2 = 0.56$, $R_{xy} = 0.76$, $P \geq 0.01$; Figure 1.1C).

The fine-scale analysis of SNP variation within Mather Field revealed a low to moderate degree of genetic differentiation among *L. fremontii* from different vernal pools within the single VP complex. In this analysis, an average of 32.00 ± 1.04 SE loci were

missing in < 8% of the genotypes across all pools, 25 loci were in HWE (Tables 1.3 and A.6), out of which an average of 13.60 ± 0.67 SE were polymorphic among pools (Table 1.3). The actual number of polymorphic loci characterizing any given pool varied between 10 and 17 (Table 1.3) due to a different number of fixed loci in each pool. At least two pools presented one private allele (Table 1.3). The average pairwise F_{ST} value between pools was 0.05 ± 0.01 SE and ranged from 0.00 to 0.13 SE (Table 1.7). While some pools were genetically indistinguishable, others were statistically distinct; one particular pool, P74, appears to be particularly differentiated from most other pools in the complex (Figure 1.2B). The first and second PCoA axes (which explain 39% and 26% of the variation, respectively) are consistent with substantial similarity among pools, though there is some tendency for clustering among nearest neighbors. The Mantel test for isolation-by-distance was not statistically significant ($R^2 = 0.01$, $R_{XY} = 0.12$, $P = 0.23$), indicating that the subtle patterns of genetic differentiation that did exist among pools are not explained by geographic distance alone.

1.4 Discussion

RADseq provided an effective technique for discovering a genome-wide set of molecular markers (SNPs) in *L. fremontii*, a wild plant species with a potentially large genome and no genomic resources available to date. My preliminary analysis of population genetic structure with these SNPs indicate that they can describe patterns of genetic variation at two spatial scales: (1) among VP complexes within the species' geographic range, and (2) among subpopulations that occupy different pools within a single vernal pool complex. Because *L. fremontii* is a dominant plant endemic to highly threatened ephemeral wetlands in California, these markers provide an important resource for ongoing efforts to design effective conservation and restoration strategies for vernal pools that promote the long-term persistence of the resident native populations.

The total number of reads obtained from the RAD library provided enough depth of coverage to identify SNP variation, even though it was relatively low compared to the number of reads that are usually expected from a lane of an Illumina Hi-seq 2500 with RAPID chemistry (Illumina, Inc.). I recovered 40% (32,084,041) of the expected high quality reads, which is lower than many studies that use RADseq (e.g. Corander et al. 2013, Reitzel et al. 2013). However, studies reporting yields <50% after quality filtering are not uncommon. For instance, Hohenlohe et al. (2011) generated ~40 million 60 bp reads prior to quality filtering from a pool of 24 individuals of cutthroat trout, of which only ~20 million were used for further analysis after filtering. Similarly, Barchi et al. (2011) obtained ~ 23 million reads from two parental individuals of eggplant prior to quality filtering and retained ~ 9 million reads for downstream analysis of an F2 segregating population. In my study, the ~60% reduction in reads was mainly due to

sequencing errors in the barcode region (barcode ambiguity), which caused those reads to be discarded by the filtering pipelines. The high barcode ambiguity rate may be due to problems in the adaptor ligation step or PCR-induced errors in the barcode sequence during library amplification (Faircloth and Glenn 2012).

Despite recovering only 40% of the expected high-quality reads, I nonetheless recovered an average of 2.67 million reads per individual that ultimately revealed 3,918 candidate SNPs. This total number of candidate SNPs is relatively high compared to other studies in plant species (e.g., Barchi et al. 2011, Xu et al. 2013). For example, an average of 2,000 SNPs were generated from a pair of mapping parents in eggplant (Barchi et al. 2011) and 3,226 SNPs were identified from 80 accessions in the cucurbit bottle gourd (Xu et al. 2013). A likely explanation for my discovery rate per consensus sequence is the high density of SNPs that I found per catalog locus. Each locus had on average 5.8 SNPs and a maximum of 22 per each of the 75 bp consensus catalog sequences, which is almost one order of magnitude higher than densities that have been reported for other Asteraceae genomes in studies using paired end sequencing. For example, one SNP per 143 bp of genome sequence was discovered in sunflower (Pegadaraju et al. 2013) and 5.6 SNPs per 1000 bp were obtained in the globe artichoke (Scaglione et al. 2012). The apparently high SNP density per catalog locus in my study is not likely to be a product of sequencing error. First, the number of SNPs per locus did not change greatly when applying different parameters for the *de novo* discovery (Figure A.1). Second, a transition to transversion ratio (T_i:T_v) of 2:1 is expected if polymorphisms were introduced at random (Petrov and Hartl 1999). The SNPs discovered in my catalog loci showed a T_i:T_v of 1.7:1, which is a reasonable ratio if

random sequencing error is not inflating my estimates of SNP variation, and is similar to that found in other Asteraceae species (e.g., T_i:T_v = 1.65:1 in eggplant; Barchi et al. 2011).

I successfully validated 44 of the 75 (62%) SNPs that I attempted to genotype, which is similar to the rates reported in other RADseq-based SNP discovery studies in plant lineages, regardless of the genotyping technology used. Karam et al. (2015) report a SNP validation rate of 50.4% using Fluidigm genotyping technology to discover SNPs in the conifer *Cedrus atlantica*, and a similar rate (50.36%) was reported for the validation step in chickpea using Illumina Golden Gate genotyping (Deokar et al. 2014).

My analysis of the validation data from a subset of the discovered SNPs revealed that even only 44 markers can successfully capture patterns of genetic differentiation among *L. fremontii* populations that occupy different VP complexes (Figure 1.1). Pairwise F_{ST} comparisons between populations in different VP complexes indicate that there is moderate to high levels of genetic differentiation at this scale, and that several populations at the range margins (specially at the southern range edge) are particularly distinct (Figure 1.1B). The northernmost population, DL, and all three southern complexes (BTM, PVP and CARR) had the highest F_{ST} values. These four outliers in the F_{ST} distribution (Figure 1.1B) contributed largely to the significant isolation-by-distance pattern observed among complexes (Figure 1.1C). Additionally, the fact that different loci had fixed alleles per complex and that the highest number of private alleles was found in the most geographically isolated complexes (NTM and CARR) suggest a high but recent degree of divergence among *L. fremontii* populations (Slatkin and Takahata 1985). In contrast, the four central complexes (BAF, JP, GT, MF) showed the least

amount of divergence (average F_{ST} = 0.028) as well as great similarity with two of the northern complexes (NTM and VINA; average F_{ST} = 0.046). These patterns are consistent with the hypothesis that there is limited gene flow among *L. fremontii* populations across large geographic scales, and particularly between the marginal populations and those at the center of the species range. Interestingly, a similar pattern of genetic differentiation and structure has previously reported in *L. burkei*, an endangered sister species of *L. fremontii* that is restricted to a small region in the Bay-Delta region of Northern California. An F_{ST} value of 0.22 was reported among *L. burkei* populations from different counties using four ISSR and two RAPD markers (see Sloop and Ayres 2010).

At local (within-complex) spatial scales, I observed variable but overall moderate degree of differentiation among *L. fremontii* subpopulations collected from different vernal pools that occurred within a total area of ~ 4 km2. This exceeds the estimates of differentiation detected by Crawford and Ornduff (1989) using allozymes in a smaller sampling of pools. These findings suggest that gene flow among pools is substantially greater than occurs at larger spatial scales (e.g., among VP complexes), but not substantial enough to homogenize subpopulations in different vernal pools within a single VP complex. I attribute patterns of population structure at this fine spatial scale to the localized foraging behavior of pollinators within pools (Emery 2009) and limited seed dispersal among pools, due in part to the island-like nature of the habitat upon which this species depends.

This study provides a novel genetic resource that can be used to quantify and describe patterns of genetic variation in a plant species that is endemic to the highly

threatened vernal pool habitat of the California Floristic Province. Overall, my results demonstrate that the RADseq approach provided an effective method for obtaining SNP markers in *L. fremontii*, which has an uncharacterized and potentially large (≥3.5GB) genome if it is similar to other Asteraceae (Lai et al. 2012, Harter et al. 2004). The availability of SNP markers, which are co-dominant and highly variable, provides a substantial advance over the markers that were previously available for this species and its closest relatives, including two *Lasthenia* species that are listed as endangered by California and federal standards (e.g., allozymes, SSRs, and sequence-based markers like nuclear ribosomal and chloroplast DNA; e.g., Crawford and Ornduff 1989, Chan et al. 2001, Ramp et al. 2006, Sloop et al. 2012). SNPs can be compared among species and genomes, and thus these resources can contribute generally to further ecological and evolutionary studies in this genus. Additionally, SNP markers are particularly valuable for conservation and management because they have the potential to provide information about the levels of homozygosity (and thus inbreeding) at the genomic and population levels, which are of particular concern for rare and threatened species (Kardos et al. 2015). My preliminary population genetic analyses, which used only 44 SNPs of the ~3800 that were discovered from the RAD library, revealed important patterns of genetic variation that can begin to inform conservation and management of this species. Most obviously, a substantial amount of the genetic diversity that currently exists within the species is currently harbored in the populations at the southern margin of the species' range. In addition to providing new information about the population genetics of *L. fremontii* in particular, these markers can also be used to obtain more general insights

into the effects of habitat loss and mitigation on the connectivity of vernal pool habitats, which can inform decisions about the conservation and management of vernal pool ecosystems in California.

Table 1.1 Names, locations, and ownership information for the vernal pool (VP) complexes from which *L. fremontii* samples were collected in this study. Rows are organized from the most northern to the most southern site. CDFW = California Department of Fish & Wildlife, USFWS = United States Fish & Wildlife Service, TNC = The Nature Conservancy, DP&R –Department of Parks & Recreation; UCNRS = University of California Natural Reserve System. Latitude and longitude are in units of decimal degrees.

Location Name (abbreviation)	Collection Year	County Name	Property Owner	Range Category	Latitude (N)	Longitude (W)
Dales Lake Ecological Reserve (DL)	2013	Tehama	CDFW	Northern	40.32878	-122.06222
Thomes Creek Ecological Reserve (TC)	2013	Tehama	CDFW	Northern	39.99493	-122.25378
North Table Mountain Ecological Reserve (NTM)	2013	Butte	CDFW	Northern	39.60068	-121.54987
Vina Plains Preserve (VINA)	2013	Tehama	TNC	Northern	39.89895	-121.98348
Beale Air Force (BAF)	2013	Yuba	United States Air Force	Center	39.60068	-121.54987
Mather Field (MF)	2013	Sacramento	Sacramento County DP&R	Center	38.53748	-121.26267
Glide Tule Ranch (GT)	2013	Yolo	CDFW	Center	38.43588	-121.6693
Jepson Prairie Preserve (JP)	2013	Solano	UCNRS	Center	38.26596	-121.82706
Arena Plains (ARE)	2008	Merced	USFWS	Southern	37.2548	-120.70986
Big Table Mountain Ecological Reserve (BTM)	2013	Fresno	CDFW	Southern	37.04526	-119.59592
Pixley Vernal Pool Preserve (PVP)	2013	Tulare	Center for Natural Lands Management	Southern	35.98547	-119.21205
Carrizo Plains National Monument (CARR)	2008	San Luis Obispo	CDFW	Southern	35.30816	-119.90955

Table 1.2 Range-wide SNP variation in 158 *L. fremontii* individuals that were collected from naturally-occurring populations. Genotypes were collected from one to four vernal pools at each site. The total number of polymorphic SNPs and at H-W equilibrium used to calculate F_{ST} values is reported per complex. The loci at Harding-Weinberg equilibrium and the number of polymorphic loci found in vernal pool complex were identified using Arlequin version 3.5.1.2 (Excoffier and Lishner 2010) when allowing 8% of missing data. The average expected (He) and observed (Ho) heterozygosity were estimated across polymorphic loci and the average number of different alleles per locus was calculated across all 44 diagnostic SNPs per VP complex.

VP complex	Pools sampled	No. individuals genotyped (No. attempted)	No. loci with < 8% missing data	No. loci at H-W equilibrium	No. poly-morphic loci	No. private alleles	Mean no. of alleles ± S.E		Ho ± S.D		He ± S.D	
DL	4	16 (20)	25	18	13	0	1.48	0.08	0.31	0.14	0.32	0.15
NTM	4	17 (20)	37	25	20	3	1.68	0.07	0.2	0.12	0.23	0.14
VINA	4	17 (19)	24	24	18	1	1.59	0.07	0.19	0.14	0.21	0.16
BAF	4	17 (20)	41	24	16	0	1.59	0.07	0.25	0.16	0.27	0.15
MF	13	16 (19)	35	21	16	0	1.52	0.08	0.26	0.18	0.29	0.16
GT	2	18 (20)	24	21	16	0	1.55	0.08	0.22	0.13	0.25	0.15
JP	4	14 (20)	35	21	14	1	1.5	0.08	0.23	0.14	0.31	0.16
BTM	2	15 (19)	32	18	14	1	1.45	0.08	0.25	0.15	0.33	0.17
PVP	4	19 (20)	31	20	14	0	1.52	0.08	0.22	0.13	0.23	0.14
CARR	1	9 (17)	10	19	12	2	1.43	0.08	0.27	0.16	0.33	0.15
Total	**42**	**158 (194)**										
Average			**29.4**	**21.1**	**15.3**		**1.53**		**0.24**		**0.28**	
SE			**2.83**	**0.8**	**0.76**		**0.024**		**0.01**		**0.01**	

Table 1.3 Fine-scale SNP variation estimated from 91 *L. fremontii* individuals sampled within the Mather Field vernal pool complex in 2011. Genotypes were collected from nine different vernal pools at the site (see Fig. 2a). The total number of polymorphic and at H-W equilibrium SNPs used to calculate F_{ST} values is reported per pool. The number of polymorphic loci by pool shown here was identified using Arlequin version 3.5.1.2 (Excoffier and Lishner 2010) allowing for 8% missing data. The average expected (He) and observed (Ho) heterozygosity was estimated across polymorphic loci per each pool, while the average number of different alleles per locus was calculated across all 44 diagnostic SNPs.

Vernal pool ID	No. individuals genotyped (No. attempted)	No. loci with < 8% missing data	No. loci at H-W equilibrium	No. poly-morphic loci	No. private alleles	Mean no. of alleles ± S.E		Ho ± S.D		He ± S.D	
E12	10 (10)	32	22	17	0	1.52	0.08	0.28	0.22	0.3	0.17
E13	10 (10)	34	19	15	0	1.45	0.08	0.22	0.17	0.29	0.14
E25	10 (10)	35	20	14	0	1.45	0.08	0.27	0.16	0.3	0.17
P10	10 (10)	30	17	13	0	1.43	0.08	0.26	0.2	0.36	0.16
P143	10 (10)	27	19	12	1	1.45	0.08	0.32	0.2	0.38	0.16
P160	10 (10)	34	21	15	0	1.5	0.08	0.19	0.18	0.32	0.15
P317	10 (10)	36	17	13	0	1.43	0.08	0.22	0.19	0.32	0.13
P318	11 (11)	32	18	13	0	1.48	0.08	0.25	0.16	0.35	0.17
P74	10 (10)	28	17	10	1	1.43	0.08	0.21	0.18	0.28	0.14
Total	**91 (91)**										
Average		**32**	**18.89**	**13.56**		**1.462**		**0.25**		**0.32**	
SE		**1.04**	**0.61**	**0.67**		**0.025**		**0.01**		**0.01**	

Table 1.4 RAD library read counts, number of putative loci after each filtering step (each row incorporates all filters above), and the final number of candidate SNPs obtained.

Step	Number of reads	Percent of reads (%)
RAD library sequencing results		
High quality raw reads obtained from library	80,726,565	100
High quality raw reads de-multiplexed	32,084,041	40
Average number of reads per individual	2,673,670	-
SNP filtering steps		
Total putative catalog loci	316,106	100
Catalog loci in > 10 individuals	754	0.24
Polymorphic catalog loci in > 10 individuals	713	0.22
Total catalog loci with ≤ 3 SNPs	238	0.07
Total number of SNPs w > 20bp flanking sequence	100	0.03
Candidate SNPs		
Total candidate	100	-
Total genotyped (KASP chemistry)	71	100
Total polymorphic among complexes	44	62

Table 1.5 Hierarchical AMOVA results for samples from the 10 VP complexes, grouped by their geographic region (northern, central and southern), among VP complexes within each region, among individuals within VP complexes, and within individuals. Fixation indices are provided below the table. Under this model, F_{SC} represents the exchange of migrants between VP complexes within a given region, while F_{CT} is the exchange of migrants between VP complexes in different regions. Taken together, these two fixation indexes gave an approximation of F_{ST} under a hierarchical island-model.

Source of variation	DF	Sum of squares	Percent variation
Among regions	2	41.3	11.9
Among VP complexes within regions	7	32.31	8.77
Among individuals within VP complexes	148	159.82	4.45
Within individuals	158	152.5	74.88

Fixation Indexes	
F_{IS}	0.06
F_{SC}	0.1
F_{CT}	0.12
F_{ST}	0.22

Table 1.6 Pairwise F_{ST} comparisons between *L. fremontii* populations in 10 vernal pool complexes that collectively span the species' geographic range (see Table 1 for complete site names that correspond to the abbreviations used here). Rows and columns are organized from the most northern (left, top) to the most southern (bottom, right) VP complex. Bias-corrected 95% confidence intervals calculated by bootstrapping are in parenthesis. Significantly different populations ($P \leq 0.05$) are indicated with an asterisk.

	DL	NTM	VINA	BAF	MF	JP	GT	BTM	CARR	PVP
DL										
NTM	0.16* (0.10 ± 0.24)									
VINA	0.09* (0.04 ± 0.15)	0.09* (0.03 ± 0.17)								
BAF	0.08* (0.04 ± 0.15)	0.09* (0.04 ± 0.16)	0.007 (-0.03 ± 0.06)							
MF	0.16* (0.10 ± 0.23)	0.12* (0.06 ± 0.20)	0.04 (-0.01 ± 0.10)	0.04 (-0.01 ± 0.10)						
JP	0.10* (0.04 ± 0.19)	0.10* (0.03 ± 0.18)	0.012 (-0.04 ± 0.09)	0.02 (-0.02 ± 0.09)	0.02* (-0.02 ± 0.10)					
GT	0.11* (0.06 ± 0.17)	0.08* (0.02 ± 0.16)	0.01 (-0.03 ± 0.07)	0.02 (-0.01 ± 0.07)	0.02* (-0.02 ± 0.09)	0.00 (-0.06 ± 0.06)				
BTM	0.19* (0.12 ± 0.30)	0.19* (0.10 ± 0.29)	0.11* (0.04 ± 0.22)	0.10* (0.03 ± 0.21)	0.10* (0.03 ± 0.21)	0.09* (0.02 ± 0.20)	0.11* (0.04 ± 0.22)			
CARR	0.29* (0.23 ± 0.38)	0.29* (0.24 ± 0.34)	0.29* (0.24 ± 0.35)	0.25* (0.21 ± 0.32)	0.29* (0.23 ± 0.36)	0.31* (0.25 ± 0.38)	0.27* (0.21 ± 0.34)	0.30* (0.23 ± 0.40)		
PVP	0.26* (0.22 ± 0.31)	0.20* (0.15 ± 0.26)	0.16* (0.13 ± 0.22)	0.16* (0.13 ± 0.20)	0.22* (0.17 ± 0.28)	0.19* (0.14 ± 0.24)	0.16* (0.13 ± 0.21)	0.21* (0.14 ± 0.31)	0.16* (0.11 ± 0.23)	

Table 1.7 Pairwise F_{ST} comparisons between nine different vernal pools within the Mather Field VP complex, developed to evaluate the distribution of genetic variation between subpopulations within a complex. Bias-corrected 95% confidence intervals, calculated by bootstrapping, are shown in parentheses. Populations that are significantly different at $P \leq 0.05$ are indicated with an asterisk.

	E12	**E13**	**E25**	**P10**	**P143**	**P160**	**P317**	**P318**	**P74**
E12									
E13	0.00 (-0.06 , 0.06)								
E25	0.00 (-0.06 , 0.07)	0.01 (-0.06 , 0.11)							
P10	0.00 (-0.07 , 0.08)	0.03 (-0.04 , 0.13)	0.01 (-0.06 , 0.10)						
P143	0.04 (-0.02 , 0.12)	0.07* (-0.01 , 0.15)	0.11* (0.03 , 0.19)	0.01 (-0.05 , 0.10)					
P160	0.00 (-0.07 , 0.07)	0.01 (-0.06 , 0.10)	0.00 (-0.07 , 0.08)	0.00 (-0.07 , 0.06)	0.03 (-0.04 , 0.12)				
P317	0.03 (-0.04 , 0.11)	0.03* (-0.03 , 0.12)	0.09* (0.01 , 0.18)	0.03 (-0.04 , 0.14)	0.05* (-0.02 , 0.13)	0.01 (-0.06 , 0.11)			
P318	0.04 (-0.04 , 0.15)	0.07* (-0.03 , 0.19)	0.09* (0.01 , 0.20)	0.03 (-0.05 , 0.12)	0.04 (-0.02 , 0.12)	0.04 (-0.04 , 0.14)	0.04 (-0.04 , 0.15)		
P74	0.04* (-0.03 , 0.14)	0.12* (0.02 , 0.24)	0.08* (-0.03 , 0.19)	0.05* (-0.04 , 0.17)	0.13* (0.04 , 0.23)	0.08* (0.00 , 0.19)	0.13* (0.04 , 0.23)	0.13 (0.03 , 0.25)	

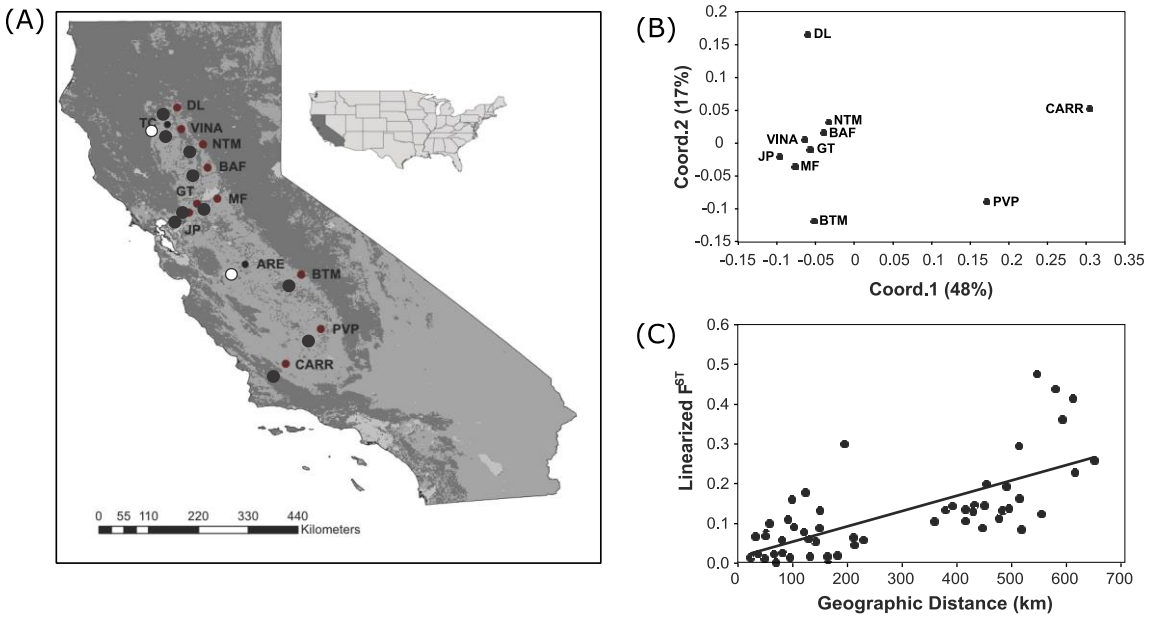

Figure 1.1 (A) The geographic locations of the 12 VP complexes from which *L. fremontii* genotypes were collected for SNP discovery. Additional samples from 10 (filled circles) of the 12 locations were genotyped to validate the SNPs. (B) Principal coordinate analysis based on the F_{ST} pairwise distances between populations from the 10 vernal pool complexes that were genotyped to validate the SNPs. (C) Results of a Mantel test of the relationship between the geographic distance and pairwise linearized F_{ST} statistics between then 10 different VP complexes ($R^2 = 0.56$, $R_{xy}=0.76$, $P \geq 0.001$).

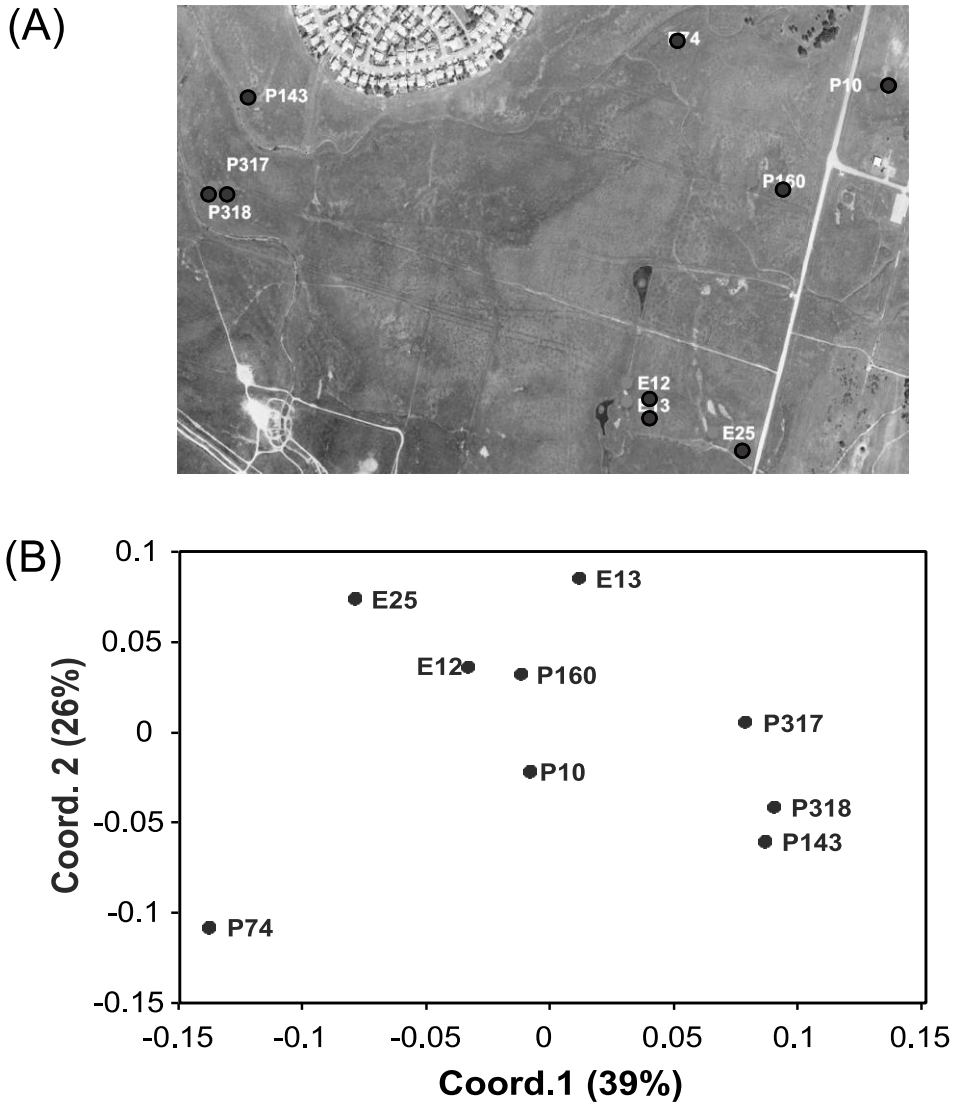

Figure 1.2 (A) Geographic positions of the nine vernal pools from which *L. fremontii* individuals were collected at Mather Field (MF in Figure 1.1A). These individuals were evaluated at 44 SNP loci to provide a preliminary analysis of the utility of these SNPs for detecting fine-scale (within-complex) population genetic structure. (B) PCo analysis based on the F_{ST} pairwise distances between the *L. fremontii* populations in the nine sampled vernal pools at Mather Field.

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CHAPTER 2. SPATIOTEMPORAL HETEROGENEITY IN PRECIPITATION PATTERNS EXPLAIN POPULATION-LEVEL GERMINATION STRATEGIES IN AN ENDEMIC SPECIALIST

2.1 Introduction

Biodiversity hotspots are found in regions with long-term climatic stability that allows population differentiation and speciation to occur across fine-scale environmental heterogeneity (Ehrlich et al. 1997). In the California Floristic Province (CA-FP) of western North America, however, the past climate has been relatively unstable compared to other biodiversity hotspots in Mediterranean regions (Cowling et al. 2015), and continues to be so today (Berg and Hall 2015, Yoon et al. 2015). In the CA-FP, edaphic habitat patches can collectively span broad climatic gradients, which can promote the earliest stage of ecological speciation—population divergence (Nosil et al., 2008; Lenormand 2012, Paun et al. 2016). In this and other biodiverse regions with strong seasonality, there is spatial variation in the extent to which climatic conditions vary within and among years (Cowling et al. 2015, Martin and Ferrer 2015). This variation can impose strong local selection on populations for key life history strategies that specifically influence persistence in temporally variable environments.

Seed dispersal and dormancy are key life history strategies that influence the persistence of plant populations in variable and unpredictable environments (Venable and

Lawlor 1980, Venable and Brown 1988). While seed dispersal allows many species to track spatiotemporal variation in suitable habitat (Clobert et al. 2012), dispersal-limited species, such as those with fragmented habitats or patchy distributions, will have difficulty shifting their distributions in response to rapid environmental change. In these species, seed dormancy can compensate for the inability to track suitable conditions through space by tracking them through time, such as by delaying seed germination until suitable conditions arise (Cohen 1966, Venable and Lawlor 1980, Donohue et al. 2010). In temperate and seasonal environments in particular, where climate is highly variable across seasons within a year, seed dormancy and germination timing can be adaptations that are critical to the survival of plant populations (Venable and Lawlor 1980, Bazzaz 2000), because they can define the environmental conditions that seedlings will experience during growth and reproduction (Donohue et al. 2010). Climate change is expected to bring increased variation, and thus decreased predictability, in climate (IPCC 2014). Seed germination traits, such as dormancy and germination time, are therefore likely to be under strong selection in the face of climate change, especially in species restricted by their habitat or with patchy distributions. Consequently, understanding natural patterns of intraspecific variation in seed germination strategies will be critical for predicting how these species will respond to climate change.

Seed dormancy is the constraint of germination in a viable seed for a period of time under conditions that otherwise would promote germination (Baskin and Baskin 2004, Finch-Savage and Leubner-Metzger 2006, Willis et al. 2014). A viable seed that germinates under a relatively wide range of conditions is considered to be non-dormant, while a narrowing of the conditions that trigger germination represents an increase in

seed dormancy (Baskin and Baskin 2004, Fernandez-Pascual et al. 2013, Baskin and Baskin 2014). It has been hypothesized that in highly variable environments dormancy serves as a bet-hedging strategy because it promotes the long-term fitness of a population by sacrificing the arithmetic mean fitness in any single year for long-term geometric mean fitness across years (Cohen 1966, Clauss and Venable 2000, Evans et al. 2007, Venable 2007). In desert annual plants, for example, dormancy has evolved as a bet-hedging strategy as a response to the variable timing of rainfall (e.g., Pake and Venable 1996, Clauss and Venable 2000, Evans et al. 2007).

Baskin and Baskin (2004, 2014) have proposed that the majority of seeds express some level of physiological dormancy that lies somewhere along a continuum, called the "dormancy continuum," between completely dormant at one extreme to non-dormant at the other. Accordingly, spatial variation in selection on dormancy across a species' range can drive differences among populations along the dormancy continuum. Population variation in dormancy has been observed along altitudinal (e.g., Beardsell and Muller 1984, Weng and Hsu 2006, Mondoni et al. 2012, Fernandez-Pascual et al. 2013) and latitudinal (e.g., Levine et al. 2008, Wagmann et al. 2012, Cochrane et al. 2015) clines. Most studies have focused on how spatial variation in dormancy reflects geographic variation in climate (e.g., Clauss and Venable 2000, Quaderi and Cavers 2002, Levine et al. 2008, Fernandez-Pascual et al. 2013) rather than historical predictability of those local conditions among populations (but see Simons 2014). However, the variability in the local environment should actually be the key factor that determines patterns of selection on dormancy levels in a population (Clauss and Venable 2000, Donohue et al. 2010), particularly if limited dispersal reduces genetic connectivity among populations.

Once a seed breaks from the dormant state, the time it takes to germinate in response to a cue (hereafter, germination time) will largely determine the conditions experienced during seedling establishment and plant growth, and thus will also be subject to selection (Donohue et al. 2010). For instance, rapid germination times are favored in *Arabidopsis thaliana* because genotypes that germinate earlier reach a larger size and have higher fecundity than those that germinate later (Donohue 2002, Donohue et al. 2010). On the other hand, slow germination time was favored due to drought stress early in the growing season of *Warea carteri*, a summer annual adapted to xeric habitats and exposed to yearly variability in precipitation conditions (Weekley et al. 2007). Consequently, temporal variation in the environment is an important driver of the adaptive evolution of germination timing in non-dormant seeds (e.g., Donohue et al, 2005).

Here I tested if there is population variation in germination strategies in *Lasthenia fremontii* (Fremont's goldfields; Madieae, Asteraceae), an annual herb that is endemic to seasonally flooded wetlands (hereafter, vernal pools) in the Central Valley of the California Floristic Province (hereafter, CA-FP). *Lasthenia fremontii* is self-incompatible and disperses its seeds by wind and gravity (Ornduff 1966, Emery 2009). Its patchy habitat, short stature, and reduced pappus (relative to its upland congeners) limit the extent to which populations can track inter-annual variation in hydrology across even local spatial scales, such as flooding gradients within pools (Emery et al. 2009). The Mediterranean climate (cool, wet winters and hot, dry summers) in this region generates annual cycles of flooding and drought in occurring depressions in the landscape. These

vernal pools harbor a large number of endemic species that are adapted to these seasonally flooded habitats. *Lasthenia fremontii* germinates from seed in the fall (usually November) with the onset of the first heavy annual rains, persists as a seedling during the winter flooding period, and then rapidly bolts, flowers, and sets seed as the water drops and temperatures rise with the arrival of spring.

The time window for germination of *L. fremontii* seeds, which occurs between the first autumn storm and the flooding of the pools, is typically quite narrow (2 – 4 weeks), but highly variable among populations that occupy different sites across the species' range. Historical climate data indicate that *L. fremontii* populations are distributed across a latitudinal gradient in precipitation, which may have imposed locally distinct (i.e., site-specific) patterns of selection on seed germination strategies. Furthermore, limited gene flow among populations across the species range (Torres-Martínez and Emery *in press*) may facilitate population differentiation due to drift and local adaptation. Here, I tested for population differentiation in the germination strategies of *L. fremontii* in response to treatments that simulated different aspects of possible germination cues, including (1) the timing of the first storm in November, (2) the amount of rainfall that the first storm brings, and (3) the length of time that water remains in the environment after the initial rain event (duration of the water cue). I characterized germination strategies in terms of two parameters, the fraction of seeds that remain dormant in response to precipitation cues (hereafter, dormancy fraction), and the time it takes for non-dormant seeds to germinate in response to precipitation cues (hereafter, germination time). I then examined whether the observed germination strategies could be predicted from historical precipitation data. Specifically, I tested if *L. fremontii* populations from locations with a

history of higher precipitation, both during the germination period and throughout the entire growing season, would exhibit faster germination because precipitation tends to be relatively abundant—and thus reliable—at those sites. In contrast, populations from sites with low levels of precipitation during the germination window may have a slower response because, once the seedling emerges from its protective seed coat, it risks encountering a dry environment that is not conducive to seedling establishment and survival. I also tested if populations from sites with historically greater inter-annual variation in precipitation during the germination period had higher levels of dormancy, which would be consistent with the evolution of locally adaptive bet-hedging strategies in response to environmental variability.

2.2 Materials and Methods

2.2.1 Study system

Lasthenia fremontii is an annual plant that is endemic to vernal pools of the CA-FP (Ornduff 1966). Vernal pools are seasonally flooded wetlands that are scattered throughout the CA-FP (Figure 2.1) and support a diverse endemic flora that are derived from primarily terrestrial ancestors (Stone 1990). The distribution of vernal pools within the region resembles island archipelagoes in a grassland ocean (Holland and Jain 1977, Zedler 2003), with individual pools clustering into vernal pool "complexes" (hereafter referred to as populations) that are patchily distributed throughout the CA-FP. Soil composition and hydrological properties vary among pools, depending on their location in the CA-FP, their position within local watersheds, and the fine-scale microtopography of individual pools (Smith and Verril 1996, Keeley and Zedler 1998, Keeler-Wolf et al. 1998, Zedler 2003). Vernal pools are temporally variable environments, both due to the annual cycle of flooding and drought (Bliss and Zedler 1998, Zedler 2003) as well as year-to-year variation in the pattern and amount of precipitation (Zedler 2003, Bauder 2005). The annual cycles of flooding and drought have allegedly favored the evolution of a uniquely adapted annual flora that can tolerate the stress of severe summer drought followed by severe flooding and even submergence, both of which can vary greatly in extent and timing each year (Bliss and Zedler 1998).

Like many vernal pool endemic plant species that are derived from non-aquatic ancestors (Stone 1990), *L. fremontii* seeds germinate in the autumn (typically in November; Bliss and Zedler 1998) with the onset of the first heavy rain of the growing season, but before standing water has developed in the pools. *Lasthenia fremontii*

populations are locally dominant on the side slopes of relatively deep vernal pools and the bottoms of shallow pools (Barbour et al. 2005, Barbour et al. 2007, Emery and Ackerly 2014). This microhabitat is characterized by particularly variable hydrological conditions. With additional rain, seedlings at sufficient depths within pools can become entirely submerged as the water table rises and remain under water for the majority of the winter season. Seedlings at intermediate depths (or in particularly shallow pools) may be repeatedly flooded and exposed as the water table fluctuates between storm events (Emery and Ackerly 2014). In spring, when precipitation declines and temperatures rise, the water table rapidly recedes and *L. fremonti* individuals bolt, flower, and set seed in the short transition period between the flooded and drought phases (Ornduff 1966, Emery 2009). Historical climate records (Worldclim database [Hijmans et al. 2014], PRISM databases [Oregon State University]) show that *L. fremontii* populations span a latitudinal gradient in the amount of precipitation they experience during the germination window in November (Figure 2.1A), but experience relatively little variation in the minimum and maximum temperatures during the same time period (Figure 2.1B). Based on these patterns, I focused on precipitation patterns as the climatic cue that may trigger different germination responses among populations from different locations within the species range.

2.2.2 Seed collection and maturation

In the spring of 2013, I collected seeds from six vernal pool complexes (hereafter called populations) that collectively spanned the species geographic range. I stratified my sampling effort to ensure balanced representation from the northern, central, and southern-most portions of the species range (Figure 2.1). Locality information,

geographic coordinates, and ownership of each vernal pool complex that I sampled were recorded (Table B.1). Within each population I randomly selected two vernal pools; in each selected pool, 30 inflorescences (maternal families) were collected and individually placed in coin envelopes containing silica gel (N = 60 maternal families per population).

In their natural environment, *L. fremontii* seeds are exposed to high temperatures and dry conditions over the summer months (roughly May – October, Figure B.1), which appear to promote seed maturation and the development of seed dormancy while attached to the maternal plant (primary dormancy; Baskin and Baskin 2004). To mimic these conditions, I exposed the field-collected seeds to hot, dry conditions by keeping them in silica gel and placing the envelopes in a growth chamber (Percival CTH-1012, Iowa, USA) set to typical California summer temperature cycles (30˚C for 15h, 15˚C for 9h) for three months prior to beginning the germination experiments.

2.2.3 Measurement of precipitation-dependent germination strategies

I generated experimental sets of 10 seeds that were randomly drawn from a pool of seeds that were collected from 60 maternal plants in a single population. Unfertilized ovules and underdeveloped seeds, both of which can be identified by their shape and color, were excluded prior to selecting each experimental set. Each set was weighed to the nearest 0.005 mg using a microbalance (Mettler Toledo XP6, Greifensee, Switzerland) to provide an estimate of seed weight that could be used as a covariate to account for maternal effects. Each set of 10 seeds was randomly assigned to one cell of a

6-well culture plate (Sigma-Aldrich, Cat No Z707759-126EA, St. Louis, Missouri, USA) lined with a single layer of 0.34 mm thick chromatography paper (3MM Chr, Whatman, Cat No 3030917).

After all seed sets were placed in their designated cell, all cell culture plates were placed in a single growth chamber (Percival CTH-1012, Perry, Iowa, USA) with temperature and photoperiod settings that approximated the minimum and maximum average values that historically characterize the Central Valley of the CA-FP in November (daylight for 10 hours at 15°C and night for 14 hours at 5°C; see Figure 2.1B). I assigned each cell to 1 of 12 possible treatment combinations that manipulated the timing (3 levels), extent (2 levels), and the duration of precipitation (2 levels) in a factorial design. The timing of the first rain event after summer (hereafter TAS) simulated early, mid, and late onset of the first major rain event each fall, which were imposed by adding water to the cells at 4, 6, or 8 weeks after seeds had been removed from summer conditions. The amount of water available to seeds from the first major rain event (hereafter WA) was manipulated by adding either 500 ul of deionized water (just enough to moisten the filter paper but not submerge the seeds) or 2000 ul of deionized water (generating standing water to simulate flooded conditions). Each TAS x WA combination was imposed for either 15 or 30 days to simulate brief or extended flooded or moistened conditions following the initial rain event (inundation length, hereafter IL). The three experimental treatments (TAS, WA, and IL) were applied using a complete factorial design, for a total of twelve different treatment combinations applied across 2,160 seeds (grouped into 216 sets of 10 seeds) representing 6 different populations of *L. fremontii*.

I evaluated the state of each seed every two days during the course of the experiment. I considered a seed to have germinated when the radicle was visible under a 10X magnifier. After recording the state of each seed in a cell as either germinated (G) or not, germinated seeds were removed from the culture plate and discarded. At the end of the experiment I evaluated every seed that did not germinate to obtain estimates of dormancy and seed mortality. To obtain an estimate of the dormancy fraction for each replicate, I dissected all seeds that did not germinate to identify those that did not have an embryo, i.e., those that were inviable from the outset of the experiment. Those lacking an embryo (NE) were counted and discarded, while those that did have an embryo were further evaluated for viability by laterally dissecting the embryos and staining them with 1% tetrazolium (TZ) overnight at 30 °C (Lakon 1948, Peters and Lanhan 2000). Ungerminated, viable seeds (i.e., those that tested positive for TZ) were used to calculate the number of seeds in the cell that were dormant (D) and those that tested negative were included in the number of seeds that had died during the experiment (M, representing the mortality fraction). Seeds that were infected with fungi during the experiment were treated as missing data (209 out of 2,160 seeds). For each experimental set I estimated the fraction of dormant seeds (FDS) out of the total number of seeds with a viable embryo, that is, FDS = D /(G +D + M). I also estimated the mean germination time of

each set as the mean number of days it took for seeds to germinate. That is, in those replicates in which germination occurred, the mean germination time (MGT) was estimated as:

$$MGT = \sum_{i=1}^{k} n_i t_i / \sum_{t=1}^{k} n_i$$

where n_i is the number of seeds germinated during the i^{th} observation or time (not the accumulated number), t_i is the time from the start of the experiment to the i^{th} observation (days), and k is the time of last germination (Ranal and Santana 2006).

I used the number of dead seeds in each set, i.e., those with an embryo that were non-viable at the end of the experiment, to calculate the mortality rate as MR = M / (G + D + M). In addition, I calculated the fraction of inviable seeds as IR = NE/10 to provide an estimate of reproductive inefficiency.

2.2.4. Data analysis of precipitation-dependent germination strategies

Differences among populations in their germination responses to simulated rainfall patterns were evaluated using a general linear model (GLM) for MGT and FDS. Each model included population, TAS, WA, and IL as main effects, and all possible interactions. The total weight of each set of 10 seeds was included as a covariate to control for differences among populations in maternal allocation to offspring. When treatments exhibited significant interactions with population, I conducted post-hoc tests to dissect the responses of each population to different treatment levels and differences among populations at each treatment level. Tukey's post-hoc comparisons were used to test for significant pairwise differences among populations. The residuals from each

analysis met the assumptions of ANOVA and so data were not transformed prior to analysis. All of the analyses were performed using PROC GLM in SAS v. 9.4.

2.2.5. Dependence of germination strategies on local rainfall conditions

I characterized the patterns of precipitation experienced by each population during the germination period using daily rainfall data for each November between 1981 and 2013 (obtained from the PRISM database, Oregon State University; Figure 2.1B). The precipitation data were extracted from the PRISM ASCII files using each population's geographic coordinates and the R-package *raster* (Hijmans et al. 2012). I quantified the year-to-year variation in precipitation during the germination period for each population as the coefficient of variation (CV) in the total rainfall each November between 1981 and 2013. Precipitation patterns for the entire winter season were also evaluated by extracting the precipitation of the wettest quarter (BIO16) and the coldest quarter (BIO19) from the Worldclim database (Hijmans et al. 2005) using DIVAgis (Hijmans et al. 2001; Figure B.2). Finally, total annual precipitation was extracted from the database to provide an estimate of the 32-year average total annual rainfall experienced by each population (Figure B.2). I used principal component analysis (PCA) to generate orthogonal climatic variables and selected the first two components (PC1 and PC2) to characterize the "germination climate niche" of *L. fremontii*. I tested for relationships between each principal component and the average values for the germination time and the dormancy fraction of each population using simple regression analysis.

2.3 Results

L. fremontii seeds germinated faster when the first rain event came later in autumn (8 weeks after removal from summer conditions) compared to earlier (4 or 6 weeks after summer; TAS effect, $F_{2, 106} = 10.44$, $P < 0.01$, Table 2.1, Figure 2.2A). In turn, the dormancy fraction was lowest when the first rain even was imposed early (4 vs. 6 and 8 weeks after summer; TAS effect, $F_{2, 137} = 10$, $P < 0.01$, Table 2.1, Figure 2.2B). When given different amounts of water during the first precipitation event, seeds germinated faster and at higher percentages under flooded than under moist conditions (WA effect, $F_{1, 106} = 70.32$, $P < 0.01$, Table 2.1, Figures 2.3C and 2.2C), with moist conditions generating greater dormancy (WA effect, $F_{1, 137} = 27.12$, $P < 0.01$, Table 2.1, Figure 2.2D). Finally, the length of time that seeds were allowed to remain in water after the first rain event (IL) also had a significant effect on germination, with seeds germinating faster when exposed to water for a shorter period of time (15 vs. 30 days) [IL effect, $F_{1, 106} = 7.89$, $P = 0.01$, Table 2.1, Figure 2.2E], and greater dormancy when they remained inundated for the longer time period (IL effect, $F_{1, 137} = 15.91$, $P < 0.01$, Table 2.1, Figure 2.2F). Overall, seeds germinated more rapidly under the conditions that maximize the time for seed development prior to germination (delayed rainfall, i.e. 8-week TAS), followed by a quick and complete imbibition of the seed coat (high volume of water, flooded WA), followed by a period of time for seedling establishment (15-day IL). Overall, dormancy was more common when the first precipitation event was early in the germination period (4-week TAS), relatively light (moist WA), and followed by an extended inundation period (30-day IL).

2.3.1 Population variation in germination responses

While *L. fremontii* populations exhibited strong overall germination responses to different levels of the experimental treatments (Table 2.1), I also observed many population-specific responses to the varying rainfall patterns tested, revealing significant intraspecific variation in germination strategies (Population effect, MGT: $F_{5, 137} = 8.83$, $P < 0.01$; FDS: $F_{5, 106} = 9.76$, $P < 0.01$; Table 2.1, Figure 2.4). Interestingly, several of these responses appeared to be associated with the position of populations within the species range. Specifically, seeds from the southernmost population (PVP) took the longest time to germinate (MGT = 12 days), while the seeds from the northernmost population (DL) germinated fastest (MGT = 5 days, Figure 2.4A). The other, more central populations had intermediate values for MGT, but with no clear pattern that corresponded to their latitudinal position within the species range. In contrast, the northernmost (PVP) and southernmost (DL) populations exhibited similar levels of dormancy (FDS) that were significantly lower than the values measured for the remaining populations (Figure 2.4B).

Populations also differed in their responses to treatment combinations, revealing different patterns of plasticity in response to the environmental conditions experienced during the germination period (Figure 2.3). For example, I observed significant differences among populations in the timing of germination (MGT) and fraction of dormant seeds (FDS) in response to the timing of the first storm event (see Population x TAS interactions in Table 2.1). The interaction between population and TAS (Table 2.3) was driven by a strong increase in the germination time of one population (BAF), under the 8-week TAS treatment level (Fig. 3A). Only seeds from BAF, one of the northern

populations, showed significantly different MGT among treatments (Table 2.3), with significantly faster germination after the summer treatment when water was introduced later (8 weeks after the summer treatment) compared to earlier (4 and 6 weeks TAS). Importantly, the variation I observed among populations in MGT did not vary consistently with latitude. I observed some differentiation in MGT between the northernmost (DL) and southernmost (PVP) population in the 4 week TAS treatment, with DL showing marginally faster germination than PVP (Figure 2.3A, Tukey post-hoc tests, $P = 0.067$, Table 2.2). However, the next most northern population (BAF) exhibited MGT values that were statistically indistinguishable from the southernmost population (PVP) and marginally significantly different from the nearest neighboring population (DL) [Figure 2.3A, Tukey post-hoc tests, $P = 0.04$]. When precipitation was introduced 6 weeks after the summer treatment ended, I once again observed significant differences between the second-northernmost and second-southernmost populations (BAF and BTM, respectively; Figure 2.3A, Tukey post-hoc test, $P= 0.02$), and between the two northernmost populations (BAF and DL; Tukey post-hoc test, $P < 0.01$). In all comparisons, BAF showed the slowest germination time, while BTM and DL showed the fastest germination times. When precipitation was introduced 8 weeks after summer ended, only DL and PVP, the northern- and southernmost populations, respectively, expressed significantly different MGT (Tukey post-hoc test, $P = 0.02$), with DL showing faster germination than PVP.

I also observed variation among populations in the extent of dormancy they expressed in response to the timing of precipitation (TAS), but the patterns were different than those observed for mean germination time (MGT) [Figure 2.3B]. Only central

populations showed significantly different levels of dormancy (i.e., the fraction of seeds that remained dormant, FDS) among TAS treatment levels (Table 2.3, Figure 2.3B), while the marginal populations (DL and PVP) and BAF (the second-northernmost) maintained similar levels of dormancy across all TAS treatment levels (Table 2.3). Both DL and PVP had the lowest proportion of dormant seeds across TAS treatments (Figure 2.3B). Differences in dormancy among populations were only observed in the 6-week TAS treatment level (Figure 2.3B). Under this treatment, the next-most-northern population (BAF) and one of the central populations, JP, had significantly less dormancy than the next-most-southern population (BTM) [Tukey post-hoc test, $P = 0.03$, $P < 0.01$, respectively; Figure 2.3B]. The two southernmost populations, BTM and PVP also showed significantly different proportions of dormant seeds (Tukey post-hoc test, $P = 0.03$), with BTM having a higher fraction of its seeds remaining dormant than PVP under this treatment level. When precipitation was introduced at either 4 or 8 weeks TAS, all populations showed levels of dormancy, ranging between 10% - 30% (Figure 2.3B).

All populations showed similar, faster germination (MGT) and lower dormancy (FDS) when the amount of water delivered during the first precipitation event (WA) was heavy compared to light (Tables 2.1 and 2.3, Figure 2.3C and 2.2D). Significant differences in MGT were observed between the two marginal populations (DL and PVP), between the two northernmost populations (DL and BAF) under the flooded treatment (Tukey post-hoc test, $P < 0.01$), and between the northernmost population and one of the central populations (DL and MF) under the moist treatment (Tukey post-hoc test, $P < 0.01$; Figure 2.3C). The northernmost population, DL had the fastest germination in these comparisons, while the southernmost population (PVP) had the slowest germination,

across both WA treatment levels (Tukey post-hoc test, P= 0.02 under the moist treatment and P = 0.01 for the flooded treatment). However, these marginal populations (DL and PVP) expressed similar low levels of dormancy under both WA treatment levels compared to the more central populations (Figure 2.3D), though overall these comparisons were not significant (Figure 2.3D, nonsignificant Population x WA interaction, Table 2.1). Under the flooded WA treatment level, I observed significantly lower dormancy in the southernmost population (PVP) compared to one central population (MF) [Tukey post-hoc test, $P < 0.01$]. However, three populations, including another southern (but not southernmost) population (BTM), a central population (MF), and a more northerly population (BAF) all exhibited significantly lower dormancy fractions than PVP in the moist WA treatment level. Thus, once again, significant population differentiation in the germination responses of *L. fremontii* varied among treatment levels, but the patterns of differences did not correspond to a simple latitudinal gradient.

All populations expressed similar, faster germination when seeds remained in water for only 15 days compared to 30 days after the initial rain event (Table 2.1, Figure 2.3E). In contrast, I observed significant differences among populations in their dormancy responses to the two levels of this treatment (Population x IL, $F_{5, 137} = 2.61$, P = 0.03; Table 2.1, Figure 2.3F). In this case, populations showed a distinct center-to-edge pattern of variation in dormancy in response to the IL treatments, with the northern- and southern-most populations (DL and PVP, respectively) having similar dormancy levels in both treatment levels, while all other populations showed greater dormancy in the 30-day IL treatment level (Figure 2.3F). Under this treatment level, the southernmost population

(PVP) had significantly lower dormancy than BTM, MF, BAF and DL, while the northernmost population (DL) only had significantly lower FDS than the next-most-northern population (BAF) in post-hoc tests that controlled for multiple comparisons.

2.3.2 Relationships between germination responses and historical local precipitation

The precipitation data used to describe historical rainfall conditions at each site over the last three decades were highly correlated (Table 2.4). When these variables were evaluated using PCA, PC1 explained 79% of the variation in the data set and was heavily weighted toward representing the amount of precipitation experienced at each site, with similarly large and positive loadings for the total precipitation in the wettest quarter, coldest quarter, calendar year, and November. The second axis, PC2, explained 18% of the variance and was most heavily loaded toward the variable representing inter-annual variation in November precipitation levels.

Simple regressions between each PC and the mean germination trait values for each population (MGT and DF) revealed that the overall level of dormancy observed in a population was significantly predicted by historical levels of variability in precipitation in November ($R^2 = 0.77$, $F_{1, 4} = 14.03$, $P = 0.02$; Figure 2.4D). Specifically, sites with higher inter-annual variation in November rainfall (i.e., larger PC2) had a larger fraction of seeds remain dormant in my experimental trials (higher FDS). I observed a weak negative relationship between the amount of precipitation historically experienced at each site (PC1) and the speed with which seeds germinated (Figure 2.4C), but this relationship was not statistically significant ($R^2 = 0.50$, $F_{1, 4} = 4.12$, $P= 0.11$), likely due to a relatively small (N=6) sample size at the population level.

2.3.3 Population variation in seed viability and mortality

In the course of evaluating seeds before, during, and after the germination experiment, I noticed substantial differences among populations in their overall seed viability and mortality rates. When seeds that did not germinate during the experiment were dissected at the end of the treatment period, I found that the proportion of seeds that lacked an embryo (inviable ratio, or IR) varied significantly among populations (Population, $F_{5, 143} = 6.54$, $P < 0.01$, Figure 2.5A). The populations from the southern portion of *L. fremontii*'s range, such as BTM and PVP, had particularly high levels of inviable seeds (29% and 32%, respectively), compared to 15-20% in all other populations (Figure 2.5A). I observed similar patterns in the fraction of seeds that had an embryo, but did not germinate, and were not alive at the end of the experiment (the mortality rate, MR, as evaluated using the TZ test; see *Chapter 2: Material and Methods*), also varied among the six populations, but these differences were only marginally significant ($F_{5, 143} = 2.03$, $P = 0.078$). Similar to the patterns for seed inviability, the highest rates of seed mortality were in the southernmost populations, with an average of approximately 38% and exceeding 40% in many replicates (Figure 2.5B). The northernmost population, DL, had particularly low rates of seed mortality, particularly compared to the southern populations. Together, the viability and mortality data both indicate that overall seed quality was generally lower in *L. fremontii* populations from the southern edge of the species in the year that my collections were conducted.

2.4 Discussion

The California Floristic Province (CA-FP) is a biodiversity hotspot characterized by historically high levels of topographic and climatic variability (Cowling et al. 2015). This environment instability generated a highly heterogeneous landscape that harbors a diverse flora of edaphic endemics that are restricted to relatively isolated, patchily-distributed habitat types (Millar 2012), including vernal pool wetlands, that are considered harsh or stressful for other organisms (Stone 1990, Kruckeberg 2006). This complex landscape promotes population divergence and local adaptation to conditions that vary among populations, such as local climate variability, which may be the earliest stages for further speciation (Lenormand 2012). Here, I tested if this process of population divergence is under way at a key life history stage for plants—germination—in a species that is endemic to CA-FP vernal pools. I predicted that I would observe divergence among populations in their responses to the different precipitation regimes that I imposed in my experiment. My results revealed significant differences among populations in both the prevalence of dormancy, and the rate at which seeds germinated in response to precipitation cues.

Inter-population variation in germination characteristics can be caused by genetic drift and local adaptation, both of which can be facilitated when gene flow among population is restricted due to patchy habitat structure (Lenormand 2012, Papaix et al. 2013). Even with relatively low levels of replication at the population level (N=6), I was able to obtain some insights into the extent to which observed differences were consistent with local adaptation by testing if germination responses could be predicted from historical precipitation data from each population's geographic location. When I

evaluated the mean MGT of each population in the context of their historical precipitation levels (PC1), I observed that germination time responded in a direction that was consistent with my prediction for adaptive differentiation: populations from sites with higher rainfall levels had faster germination (Figure 2.4C). However, this result was not statistically significant, possibly due to a sample size of 6 for testing this relationship, and the high variance in MGT within populations due to the numerous precipitation treatments that were imposed in the experiment. Nonetheless, MGT is clearly different among populations (Figure 2.4A) and the trend towards faster MGT in wetter sites is consistent with the hypothesis that this divergence may be due to local adaptation to precipitation levels in different habitats. I observed stronger support for my prediction that populations from locations with historically high variability in precipitation would have experienced selection favoring dormancy (Figure 2.3D). Specifically, I observed a statistically significant relationship between the variability in autumn precipitation (PC2, Table 2.4) from each site and the mean dormancy fraction (FDS) of the resident population (Figure 2.3D). This result indicates that the variability in the amount of rainfall, rather than the absolute amount of rainfall, experienced by a population during the germination window explains inter-population differences in dormancy.

The populations from locations that have relatively low variability in autumn rainfall, and correspondingly low levels of dormancy, occurred at the extreme northern and southern edges of *L. fremontii*'s geographic range (Figure 2.4B). One hypothesis for the evolution of range limits is that range edges represent the limits of the species' niche, and thus are relatively stressful environments that will lower mean fitness and population growth rates relative to those that will occur nearer the range center. The reduced growth

rates may, in turn, generate asymmetric gene flow from the range center to the range edge, limiting the potential for marginal populations to adapt to their local environments (Haldane 1956, Antonovics 1976, Kirkpatrick and Barton 1997). The results from this study contradict this scenario in two important ways. First, *L. fremontii* populations have differentiated across the species' range, particularly the marginal populations, as many of the significant pairwise comparisons between populations included at least one marginal population (DL or PVP; see *Chapter 2: Results*). At least one response—the dormancy fraction—has diverged in a direction that is consistent with adaptation to reduced variation in the environment (Figure 2.4D). Second, the reduction in dormancy at the range edge could lead to higher population growth rates, rather than lower, and possibly counter the effects of antagonistic gene flow due to differences in population size or mean fitness (Venable 2007, Evans et al. 2007). Dormancy is not favored in temporally constant environments (Baskin and Baskin 2004), as occurs at the northern and southern range edges in *L. fremontii* (Figures 2.4B and D). Instead, selection may have favored precise germination timing (MGT) in these populations (Figure 2.4C) that minimizes the stress experienced by seedlings during early establishment. In the northernmost population (DL), precipitation is consistently high each year (Figure 2.1B and 2.4D), so there is little risk of seedlings experiencing drought after emergence. In this environment, earlier and faster germination may provide a competitive advantage later in the life cycle and a longer reproductive season (as in Donohue 2002, Donohue 2010). In contrast, the southernmost population (PVP) is from the site with the lowest average annual rainfall (Figure 2.1B), so plants that germinate early could face a high risk of early mortality due to dessication (as in Weekley et al. 2007). In these drier environments, delayed

germination may increase the probability that there will be sufficient precipitation available for juvenile survivorship early in the life cycle, when roots are establishing and seedlings are particularly sensitive to the amount and frequency of rainfall events.

The *L. fremontii* populations near the center of the species' range exhibited remarkably similar germination strategies (GMT and FDS) despite spanning a latitudinal gradient in precipitation. These populations exhibited evidence of both bet-hedging dormancy and accurate germination timing in their overall germination strategies (Figures 2.4B and D). However, all of these *L. fremontii* populations typically experienced low year-to-year variability in the amount of precipitation they experience during the November germination period, especially in comparison with the marginal populations (Figure 2.4D). Consequently, their responses reinforce the importance that selection by temporal variation in precipitation, rather than the absolute amount of precipitation, appears to have a stronger effect on the differences in local germination strategies observed across the geographic range of this narrowly distributed vernal pool species.

The importance of temporal variation in driving the divergence of germination strategies among *L. fremontii* populations is further emphasized by the extent of plasticity I observed in response to many of the precipitation regimes I imposed in the experiment (Figure 2.3). All populations, with exception of PVP and BAF, exhibited faster germination when precipitation was introduced late (8 weeks after the summer), which could serve as a cue for a short growing season ahead. In some cases, population differences were evident in only some levels within a treatment, suggesting that populations may vary in their extent of plasticity in germination. For example, significant

differences among populations in MGT were observed in the 4-week and 6-week TAS levels, but not in the 8-week treatment level (Figure 2.3A). Furthermore, PVP, DL and BAF showed relatively constant levels of dormancy across all three TAS treatment levels, while several other populations exhibited a strong peak in dormancy at the 6 week treatment level (Figure 2.3B).

The results of this study contribute to a growing body of evidence showing relatively consistent germination responses of California vernal pool endemic plant species to the timing of autumn rains. Bliss and Zedler (1998) experimentally demonstrated that several vernal pool endemics and wetland generalists had higher germination percentages (and thus lower dormancy rates) under treatments that simulated earlier autumn rain events, which is the same overall pattern that I observed in *L. fremontii* (Figure 2.2). Seed dormancy has also been documented in several vernal pool endemics, including *Orcuttia spp.* (Griggs and Jain 1980), *Limnanthes alba* (Cheng and Gordon 2000), and *Pogogyne abramsii* (Zammit and Zedler 1990), purportedly as a mechanism for persisting in the highly variable environment of vernal pools. However, to my knowledge, mine is the first study to test for intraspecific variation in the germination strategies among population of a vernal pool endemic species. Because my results indicate that germination strategies can vary substantially among populations within individual species, further studies of intraspecific variation in germination characteristics are warranted in other vernal pool taxa.

The presence of population variation in germination strategies of edaphic endemic species likely has important consequences for how these species' will respond to future climate change. Importantly, the current germination responses in *L. fremontii*

populations may, at least in some cases, reflect local adaptations to historical patterns of climatic variability (e.g., Figure 2.4D). Like other patchily-distributed, edaphic endemic plant species in the California Floristic Province, *L. fremontii* may not be able to disperse rapidly enough to track climate change, which is predicted to reach a particularly high velocity in the Central Valley of California over the 21st century (Loarie et al. 2009). Consequently, seed traits that regulate the germination process will likely play a key role defining their responses to climate change by tracking their optimal climates through time. In California, climate change is projected to increase annual variation in rainfall conditions over the next century, with extremely dry and wet seasons becoming 1.5 to 2 times more common (Berg and Hall 2015, Wang et al. 2015). Given the variation in germination strategies observed among *L. fremontii* populations, I expect that seed dormancy will be the main trait to buffer the projected climate variation. However, the consistently wet and dry conditions historically experienced by the northern and southern peripheral populations, respectively, are projected to be maintained, though perhaps at more extreme average levels (Berg and Hall 2015). These peripheral populations currently express the lowest levels of dormancy as and possibly the most precise germination times for their historical climates (Figure 2.4), suggesting that these populations may be particularly prone to extinction under the projected patterns of climate change. The southernmost population may be particularly susceptible to the projected extreme drought events (Wang et al. 2015) that would eliminate the vernal pool environment altogether. My observation that the southernmost populations already express a lower proportion of viable seeds (Figure 2.5) further emphasizes the threat to this portion of the species range.

As highlighted in this special issue, the climatic history of biodiversity hotspots is central to explanations for the generation of and maintenance of species diversity in floristically diverse regions. Furthermore, the climatic history of these regions has driven the evolution of life histories in the resident plant species that will critically define their responses to human-driven climate change. My work shows how fine-scale heterogeneity in edaphic conditions can interact with large-scale spatiotemporal variation in climate to promote population differentiation in life history traits of edaphic specialists. The results suggest that relatively subtle differences in climate variability experienced by populations in different habitat patches may drive the divergent evolution of life history traits in germination and dormancy characteristics. These traits, in turn, will have important consequences for population persistence in the facing of increasingly variable climates projected for the near future.

Table 2.1 Results from an ANOVA model evaluating the effects of population and all precipitation treatments on the mean germination time (MGT) and dormancy fraction (FDS) in response to different precipitation regimes. Seed weight was included as a covariate to account for maternal effects. The overall model for each variable was statistically significant (MGT: df=69, MS=38.68, F=3.87, P=0.01, R^2=0.72; DR: df=72, MS=0.10, F=3.36, P<0.01, R^2=0.72). TAS = time of precipitation addition after summer treatment ended (3 levels); WA = amount of water added at watering event (2 levels); IL = inundation length following watering event (2 levels).

	MGT				DR			
Source	**df**	**MS**	**F**	***P***	**df**	**MS**	**F**	***P***
Population	5	97.57	9.76	<0.01	5	0.27	8.83	<0.01
TAS	2	104.34	10.44	<0.01	2	0.3	10	<0.01
Population*TAS	10	23.05	2.31	0.02	10	0.06	2.11	0.03
WA	1	702.99	70.32	<0.01	1	0.82	27.12	<0.01
Population*WA	5	9.53	0.95	0.45	5	0.02	0.56	0.73
WA*TAS	2	5.08	0.51	0.6	2	0.01	0.31	0.73
Population*WA*TAS	10	29.99	3	0	10	0.02	0.65	0.77
IL	1	78.91	7.89	0.01	1	0.48	15.91	0
Population*IL	5	0.47	0.05	1	5	0.08	2.61	0.03
WA*IL	1	1.61	0.16	0.69	1	0.05	1.68	0.2
Population*WA*IL	5	4.38	0.44	0.82	5	0.06	1.83	0.11
TAS*IL	2	0.85	0.09	0.92	2	0.27	9.04	0
Population*TAS*IL	10	10.03	1	0.45	10	0.07	2.27	0.02
WA*TAS*IL	2	2.39	0.24	0.79	2	0.22	7.39	0
Population*WA*TAS*IL	7	9.48	0.95	0.47	10	0.04	1.41	0.18
Seed weight	1	0.23	0.02	0.88	1	0	0.05	0.83

Table 2.2 Post-hoc tests for differences among populations within treatments. These tests were conducted only for significant population x treatment interactions that were found in ANOVA models evaluating the effects of population identity and water treatments on the mean germination time (MGT) and fraction of dormant seeds (FDS) (see *Table 2.1*). The comparisons in this table tested for significant differences among populations within each level of each treatment. TAS = time of precipitation addition after summer treatment ended (3 levels); IL = inundation length following watering event (2 levels).

			MGT			FDS		
Treatment	**Level**	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**
TAS	4 weeks	5	62.25	3.9	<0.01	0.08	2.55	0.03
	6 weeks	5	75.87	4.76	<0.01	0.22	7.17	<0.01
	8 weeks	5	53.29	3.34	0.01	0.08	2.75	0.02
IL	15 days	5				0.09	2.83	0.02
	30 days	5				0.25	8.12	<0.01

Table 2.3 Post-hoc tests of treatment effects within populations when the population x treatment interaction was significant in ANOVA models evaluating the effects of population identity and water treatments on the mean germination time (MGT) and fraction of dormant seeds (FDS) (see Table 1). The comparisons in this table reflect tests for differences among treatments within each population. TAS = time of precipitation addition after summer treatment ended (3 levels); IL = inundation length following watering event (2 levels).

			MGT			FDS		
Treatment	**Population**	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**
TAS	DL	2	6.6	0.41	0.66	0.03	1.1	0.33
	BAF	2	100.68	6.31	<0.01	0	0.05	0.96
	MF	2	38.34	2.4	0.09	0.18	5.89	<0.01
	JP	2	41.63	2.61	0.08	0.14	4.52	0.01
	BTM	2	35.86	2.25	0.11	0.3	9.96	<0.01
	PVP	2	13.34	0.84	0.44	0	0.01	0.99
IL	DL	1				0	0.15	0.7
	BAF	1				0.55	18.14	<0.01
	MF	1				0.25	8.2	<0.01
	JP	1				0.05	1.79	0.18
	BTM	1				0.05	1.71	0.19
	PVP	1				0	0.04	0.85

Table 2.4 The first and second eigenvectors, and respective eigenvalues and loadings, generated from a Principal Components Analysis (PCA) using precipitation data from the PRISM and Worldclim databases to characterize the precipitation regime at the geographic location of each *L. fremontii* population evaluated in the germination experiment.

Variable	PC1 79%	PC2 13%
Precipitation of the wettest quarter (BIO16)	0.481	0.272
Precipitation of the coldest quarter (BIO19)	0.483	0.216
Annual precipitation	0.5	0.006
November precipitation	0.491	-0.086
Variation in November precipitation (CV)	-0.209	0.934

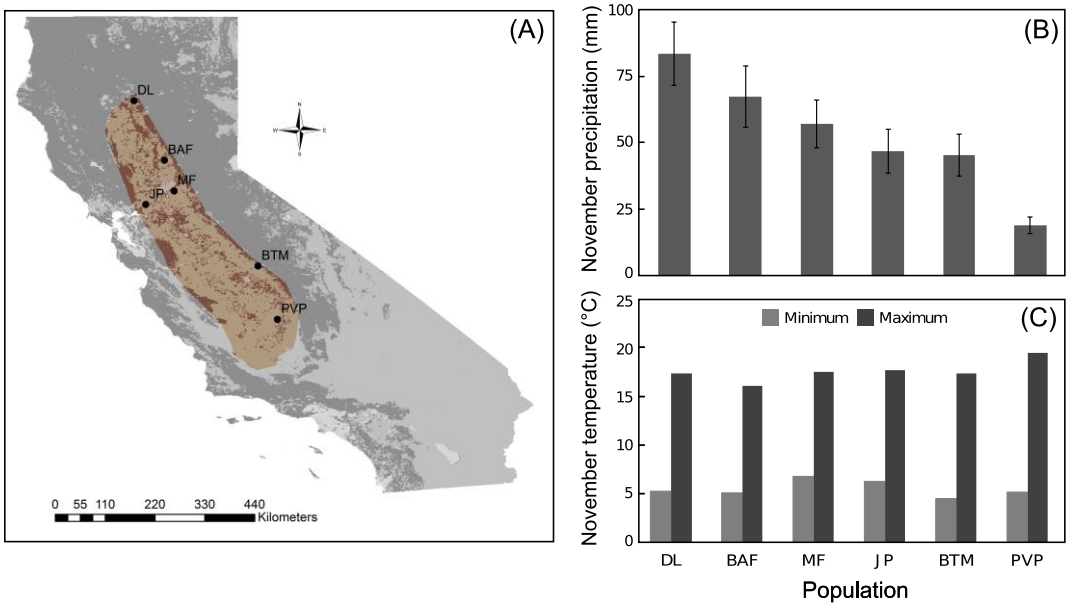

Figure 2.1 (A) The geographic locations of the six populations that were represented in the seed germination experiment, which collectively span the geographic range (shaded area) of *L. fremontii* in the Central Valley of the California Floristic Province. (B) The 32-year average (± 1 SE) for total precipitation in November (the germination period for *L. fremontii*) at the locations corresponding to each population included in the experiment (generated from daily precipitation data obtained from the PRISM database). (C) The 32-year averages for the minimum and maximum temperature in November for the geographic position of each population (obtained from interpolated climate data from the Worldclim database).

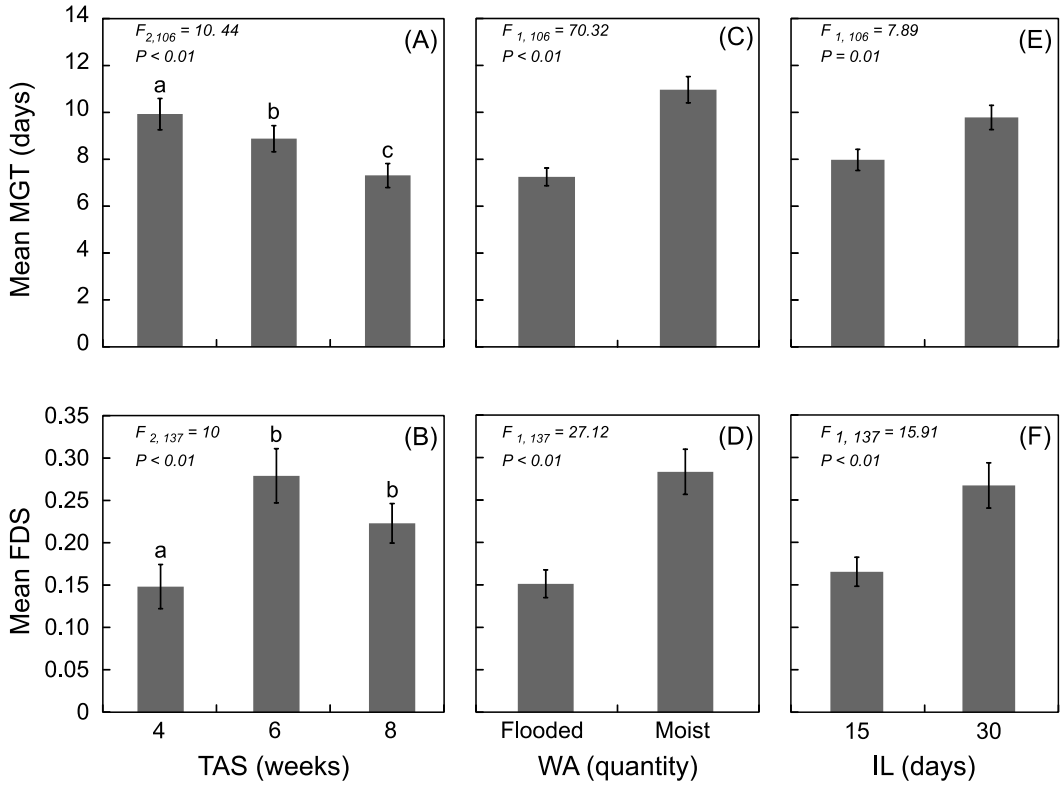

Figure 2.2 *L. fremontii* germination responses overall populations in terms of the mean germination time (MGT; top row) and fraction of dormant seeds (FDS; bottom row) to variable precipitation patterns during the germination window (November): (A, B) the timing of the first rain event (TAS) at 4, 6, or 8 weeks after the summer treatment ended; (C, D) the amount of the first rain event (WA), moist or flooded; and (E, F) the length of inundation (IL) following the initial rain event, 30 or 15 days. Error bars represent 1 standard error.

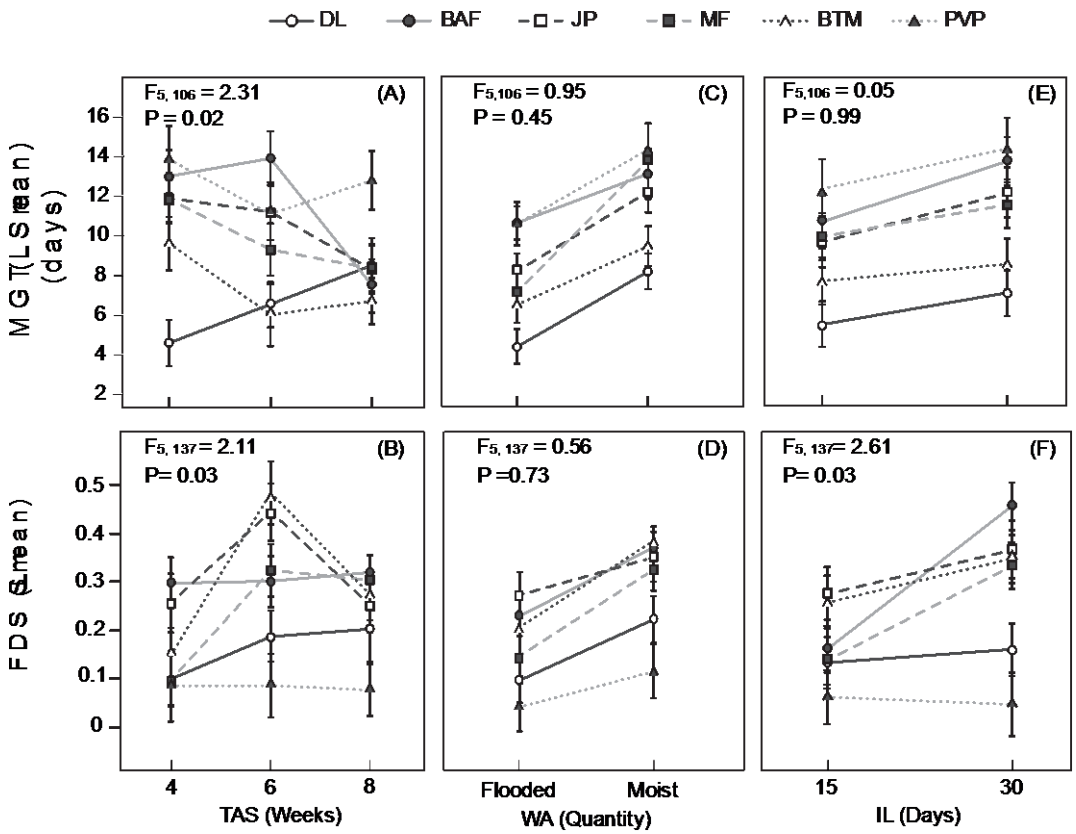

Figure 2.3 Reaction norms showing how population mean germination time (MGT; top row) and fraction of dormant seeds (FDS; bottom row) vary among levels of three different precipitation treatments: (A, B) the timing of the first rain event (TAS) at 4, 6, or 8 weeks after the summer treatment ended; (C, D) the amount of the first rain event (WA), moist or flooded; and (E, F) the length of inundation (IL) following the initial rain event, 30 or 15 days. Error bars represent 1 standard error.

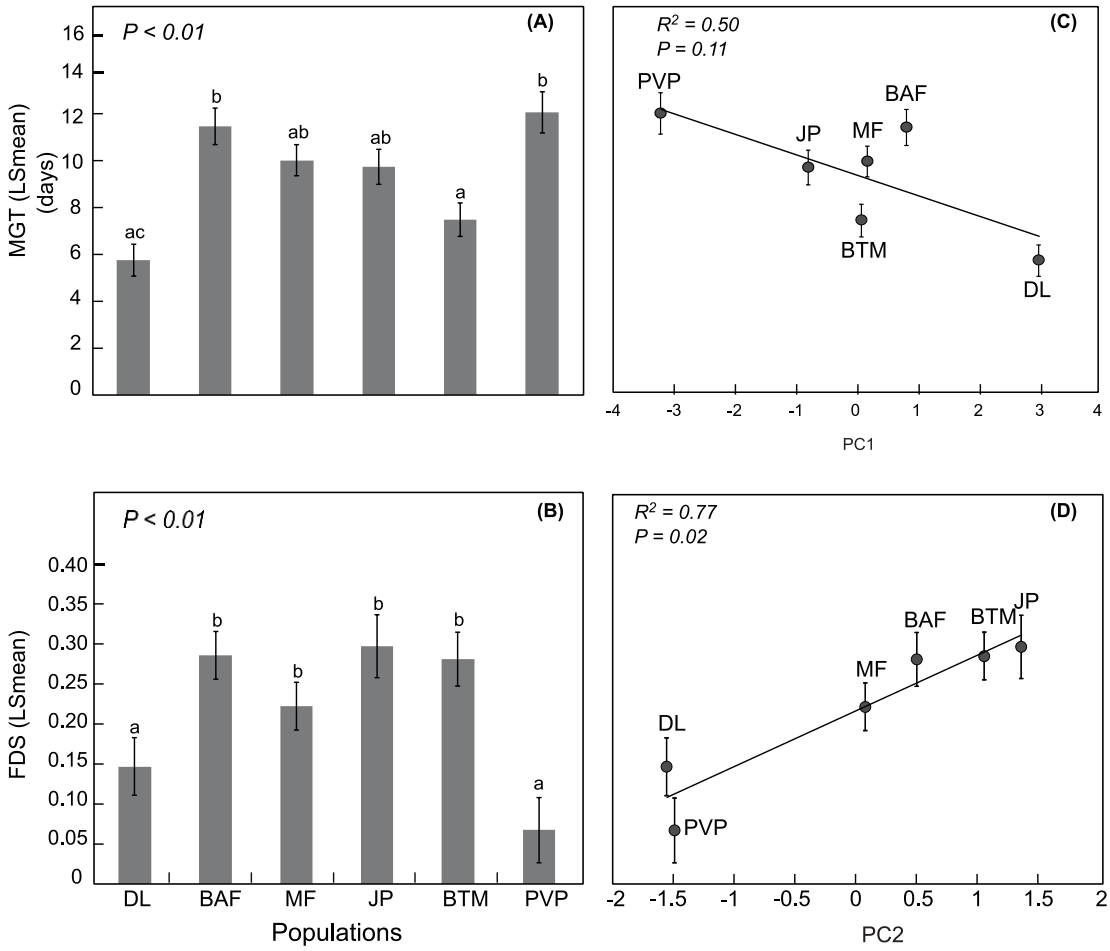

Figure 2.4 Population variation in germination strategies and relationships with historical precipitation data. (A) and (B): Population LSmeans (± 1SE) for the mean germination time (MGT) and fraction of seeds remaining dormant (FDS), respectively, observed in the seed germination experiment, averaged across all precipitation treatments. Letters above bars identify statistically significant differences between means using Tukey's *post hoc* tests to control for multiple comparisons. Populations are are arranged on the x-axis by latitude, from the most northern (left) to southern (right). (C) A weak negative relationship was observed between the amount of precipitation characterizing each population's geographic location (PC1) and its grand mean germination time (± 1SE), suggesting that populations from drier sites tend to take longer to germinate in response to the initial precipitation cue during the germination period, though this result was not statistically significant. (D) A significant positive relationship was observed between a variable that captured the interannual variability in precipitation (PC2) and the prevalence of seed dormancy observed in the experiment (FDS), indicating that populations from sites with historically more variable precipitation levels during the germination window maintain a higher level of dormancy.

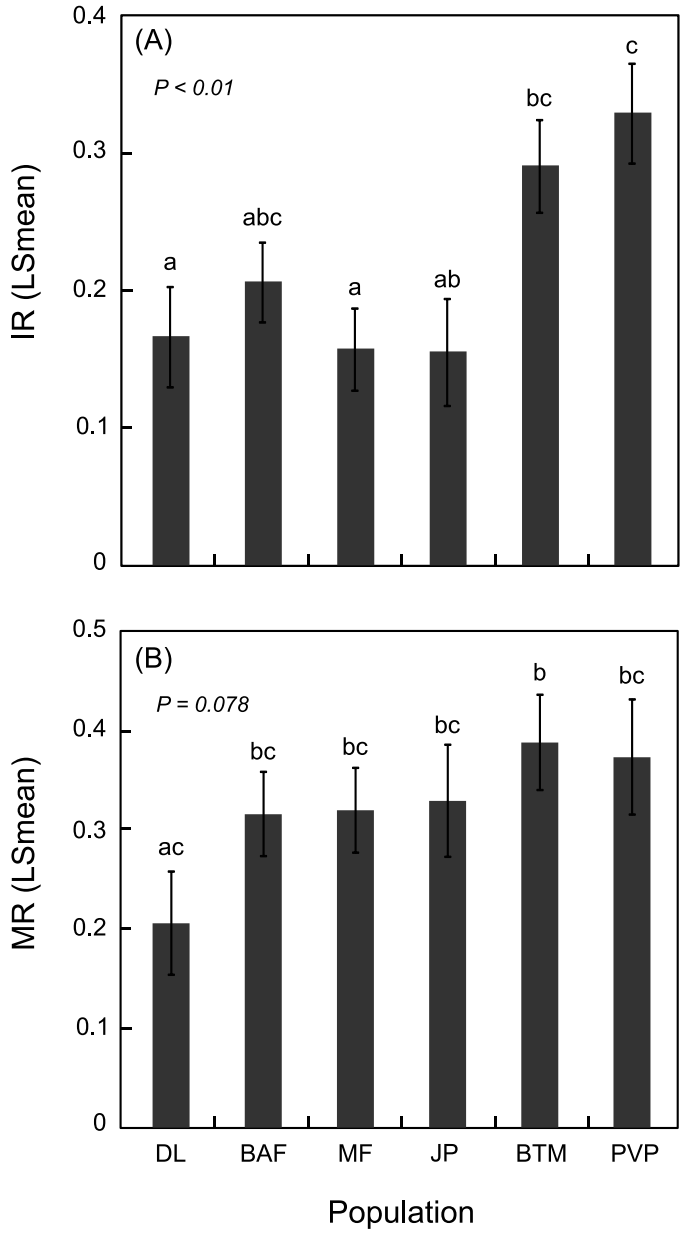

Figure 2.5 Variation among populations in the proportion of seeds from each population that (A) lacked an embryo (NE), or (B) had an inviable embryo, when seeds that did not germinate in the experiment were dissected at the end of the experiment. Bars represent population LSmeans, averaged across all experimental treatments, ± 1SE.

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CHAPTER 3: EVOLUTIONARY POTENTIAL OF *L. FREMONTII* IN RESPONSE TO EXTREME CLIMATE EVENTS

3.1 Introduction

One of the most challenging tasks facing scientists today is to predict the potential for species to respond to novel climate regimes and other sources of rapid environmental change due to human activities. The potential for species to respond to a changing climate through range shifts is limited in species that depend on naturally patchy or recently fragmented habitats, which reduce the opportunity for populations to track climate change through dispersal (Etterson 2008, Hoffmann and Sgro 2011) In these cases, species persistence will depend heavily on the potential for populations to adapt *in situ* to novel climate regimes (Lenoir et al. 2008, Parmesan 2006, Thuiller et al. 2011), particularly in response to selection for tolerating more extreme and variable climates. This potential will be determined by their patterns of adaptation to local historical climate regimes (Kelly et al. 2013), as well as the amount of genetic variation harbored within populations for responding to changing climatic conditions (Gomulkiewicz et al. 2010, Hoffmann and Merila 1999, Shaw and Shaw 2014).

Patterns of local adaptation to current climate will heavily influence how individual populations will respond to climate change, especially to extreme and novel climate regimes. When a species' range spans diverse and heterogeneous conditions,

contrasting selective pressures can promote local adaptation and genetic differentiation among populations (Agren and Schemske 2012, Etterson 2004, Kelly et al. 2013). Local adaptation to past climate will be particularly likely to exist in species that are composed of patchily-distributed, geographically isolated populations because reduced gene flow among populations will have facilitated evolutionary responses to local selective pressures (Davis et al. 2005, Hoffmann et al. 2005). The degree of adaptation to local historical climate will determine a population's short-term demographic response to new climate regimes. For example, new climatic conditions that are far from a population's optimum will cause an immediate reduction in population size and mean absolute fitness ($\bar{W}$), increasing its susceptibility to the effects of drift and inbreeding (Franks et al. 2014, Kim and Donohue 2013, Shaw and Etterson 2012). In this way, current patterns of adaptation set the stage for future evolutionary responses within populations by determining their short-term demographic persistence under strong and rapid changes in selection. Thus, understanding these patterns on populations of patchily-distributed species is the first step in assessing their potential for responding to new climate regimes.

If a population persists when its environment changes, its potential to adapt to new conditions depends on the amount and distribution of additive genetic variation that remains in the population. According to Fisher's Fundamental Theorem of Natural Selection, "The rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time'' (Fisher 1930). Based on this framework, the evolutionary potential of a population (i.e., its capacity to adapt in response to new selective pressures) can be measured as the additive genetic variation in fitness that is observed in the population under those conditions. Shaw and Shaw (2014) proposed that

the ratio of additive genetic variation in absolute fitness, hereafter $V_A(W)$, and mean absolute fitness $(\bar{W})$ can predict the increase in population mean fitness after one generation of selection, or the rate of adaptation. Thus, high $V_A(W)$ under new conditions would indicate that the rate of adaptation of a population under novel climate conditions will also be high, particularly when the population is far from its optimum (i.e., has low $\bar{W}$) [Shaw and Etterson 2012]. The genetic variation in fitness that a population will express under novel conditions is largely determined by the history of gene flow, genetic drift and past selection pressures that have operated within the population (Etterson 2008, Lopez et al. 2008, Shaw and Etterson 2012). Recently, Kellermann et al. (2009) demonstrated that narrowly distributed species of *Drosophila* that are restricted to tropical conditions had lower V_A in desiccation and cold resistance traits compared to more widely-distributed species. Thus, the $V_A(W)$ expressed under novel climate regimes should be expected to be different among discrete populations due to their unique recent evolutionary histories of selection, drift and gene flow. Yet, few studies (e.g., Kelly et al. 2013) have validated these expectations in the face of extreme climatic conditions.

Recently there has been growing recognition that the expression of genetic variation can also vary with environmental context (Hoffmann and Merila 1999, McGuigan and Sgro 2009, Sgro and Hoffmann 1998). Just as the expression of a genetically-based trait can depend on the environment (G x E), so can the genetic variation that is expressed (hereafter V_G x E). Several experimental studies have found that populations may express genetic variation in a novel or stressful environment that was not evident in the typical environment (Charmantier and Garant 2005, Hoffmann and Merila 1999, Hoffmann and Parsons 1991, Imasheva et al. 1998, Schlichting 2008).

These data have prompted the hypothesis that heritability is higher under novel or stressful conditions due to an increased in the expression of V_A (Hoffmann and Merila 1999, Imasheva et al. 1998). However, other studies have shown that the expression of genetic variation can increase when conditions become more benign (e.g., Emery and Ackerly 2014, Kristensen et al. 2015). In general, the direction of the response of V_A in a population when its environment changes is still the subject of much discussion (McGuigan and Sgro 2009), but the fact that it *does* change is becoming recognized as a common phenomenon that can have an important influence on the potential for adaptive evolution in different environments. Importantly, the effects of V_G x E on population's responses to novel climates are almost entirely unexplored, despite its key role in the capacity for populations to evolutionary respond to climate change. Recent simulations of V_A under changing environmental conditions predict that an increase in additive genetic variation in fitness, but a decline in the mean population's fitness, can be observed as a direct effect of a changed environment (Shaw and Shaw 2014). In these models, the cumulative effect was an increase in the population's adaptive potential due to the increase in the ratio $V_A/(\overline{W})$, although a further environmental change in the following generation could counteract this increase by modifying $(\overline{W})$. Empirical studies that explicitly evaluate the direction in which V_A will change under projected climate regimes are needed to accurately predict the evolutionary implications of climate change for populations.

The goal of this study was to investigate the evolutionary potential for populations of a patchily distributed plant species to respond to the extreme climate regimes that are projected to develop at their home sites over the next century. Specifically, I asked the following questions:

(1) Do the effects of climatic extremes on mean fitness vary among populations that have historically experienced different climates?

(2) Do populations vary in their expression of additive genetic variation in fitness (i.e., their evolutionary potential) in response to extreme climate conditions?

(3) Does the expression of additive genetic variation in fitness change with the climate experienced by a population?

I addressed these questions by conducting a greenhouse experiment that evaluated the effects of precipitation conditions on fitness in *Lasthenia fremontii* (Madieae, Asteraceae), an annual herb that is endemic to vernal pool wetlands of the Central Valley of California (USA). Vernal pools are relatively small, shallow depressions in the landscape that lie above an impermeable soil type horizon called hardpan (Smith and Verrill 1996, Chetham 1976, Weitkamp et al. 1996). In the California Floristic Province of western North America, vernal pools were common in the Great Central Valley prior to European settlement, though today they are considered a heavily threatened habitat type due to urban development and agricultural expansion. The Mediterranean climate of this region generates annual cycles of flooded and dry conditions within pools, as the pools fill with water with the winter rains, gradually dry during the spring, and remain dry throughout the summer (Holland 1981, Zedler 2003). Thus, in California these habitats are heavily dependent on precipitation as the driving source of water that

accumulates each winter, with evapotranspiration as the main process of water loss in the spring (Hanes 1996, Pyke 2004). Hydrological modeling of vernal pools has shown that the vernal pool hydroperiod—the number of days a vernal pool is flooded—is first determined by the local rainfall conditions in a specific region and then modified by environmental factors that can vary among locations or even individual pools (e.g., soil, bathymetry, topography, landscape position) [Pyke 2004, Bauder 2005]. This makes it possible to impose realistic experimental treatments that simulate the precipitation patterns that are projected by climate change by simply manipulating the hydroperiod experienced by plants grown under otherwise standardized conditions.

Here I evaluate the evolutionary potential of *L. fremontii* in three populations from geographic locations that have historically experienced different levels of annual precipitation (and thus vernal pool hydrology). In California, extreme precipitation events are expected to increase over the next century due to the projected increase in frequency and intensity of El Niño and La Niña events (Berg and Hall 2015, Cayan et al. 2008, Yoon et al. 2015), and so I was particularly interested in examining the effects of precipitation extremes on the expression of additive genetic variation in fitness in different populations. First, I tested if populations vary in their overall fitness responses to alternative precipitation conditions. I hypothesized that latitudinal differences in precipitation across California and low gene flow among populations have driven divergence in climatic tolerances among populations across the species range. Based on this hypothesis, I predicted that populations would vary in the effects of climatic extremes (particularly short or long hydroperiods) on mean fitness ($\bar{W}$). Furthermore, if populations have locally adapted to their "home" precipitation patterns, I would expect

their mean fitness to be relatively resistant to climatic extremes that are similar to conditions they have experienced in their recent histories. Second, I tested for differences in the genetic variation in fitness [i.e., evolutionary potential, $V_A(W)$] among populations under normal and extreme precipitation conditions. Here, I hypothesized that genetic variation in fitness will differ among populations due to their unique evolutionary histories of selection, drift, and intra-population gene flow. Finally, I tested if the expression of additive genetic variation in the population varies with climatic conditions (i.e., if $V_A(W)$ changes with the length of the hydroperiod). This question is particularly important because it addresses the extent to which the evolutionary potential of populations is a function of the climate itself. Based on previous studies, I hypothesized that $V_A(W)$ would vary across my experimental treatments, but I had no *a priori* expectation for the direction of change (increase or decrease) I would observe under extreme vs. "normal" hydrological conditions.

3.2 Materials and Methods

3.2.1 Study system

Lasthenia fremontii is an annual plant species that is endemic to the vernal pool wetlands of the California Floristic Province (Ornduff 1966, Emery et al. 2011; Figure 3.1A). Vernal pool wetlands are, by nature, highly variable environments that annually cycle between flooded and dry stages. The pools usually fill with water with the winter rains, gradually dry during the spring, and remain dry through the summer (Holland and Jain 1981, Zedler 2003). The hydrological dynamics of an individual pool (e.g., length of hydroperiod, maximum depth, fluctuations in the water table, and rate of filling and drying) vary substantially among years, depending on the amount and patterns of precipitation that occur over the wet winter season (Bauder 2005, Emery et al. 2009). Vernal pools occupy a range of edaphic and climate conditions in the California Floristic Province, which generates spatial heterogeneity among pools that occupy different positions within the landscape or across the region.

Vernal pools that support *L. fremontii* populations are distributed throughout the Great Central Valley. Like many vernal pool endemics, *L. fremontii* exhibits a life cycle that allows it to persist despite the extreme stresses of inundation and drought that characterize vernal pools in this region (Bliss and Zedler 1998). Seeds of *L. fremontii* germinate each autumn (typically in November) when the first major storms saturate the soil but prior to submergence. Further rain eventually submerges the seedlings, which remain under water for the rest of the wet season. Further above-ground growth and flowering is delayed until the following spring when the pools start to dry (Ornduff 1966, Emery 2009). Individual plants quickly bolt, reproduce, and disperse their seeds during

the brief waterlogged period, prior to onset of the hot, drought-like conditions that characterize the Central Valley in the summer. Populations from northern, central, and southern positions within the species range span a gradient in historical total annual precipitation that is lowest in the south and highest in the north (Data extracted from Worlclim database [Hijmans et al. 2005], Figure 3.1B). In contrast, temperature is relatively similar among sites from these different locations within the species range (Data extracted from Worlclim database [Hijmans et al. 2005], Figure 3.1C).

3.2.2 Seed collection from source population

I selected three vernal pool complexes that represented a population from the northern edge (Dales Lake Ecological Reserve, hereafter DL), southern edge (Pixley Vernal Pool Preserve, hereafter PVP), and center (Mather Field, hereafter MF) of *L. fremontii*'s geographic range (Figure 3.1A, Table 3.1). At each location, I collected all seeds from 50 plants in each of four randomly-selected vernal pools, for a total of 200 maternal families collected per site. Each seed sample was placed in a labeled coin envelope containing silica gel to prevent moisture accumulation. The envelopes were mailed to Purdue University and stored for two weeks in laboratory conditions at 21°C (similar to spring temperatures of California), and then placed in a growth chamber (Percival CTH-1012, Iowa, USA) that was programmed to simulate summer conditions (30˚C for 15h, 15˚C for 9h) for three months. This pre-experimental treatment was imposed to promote seed maturation and development (Ornduff 1966).

3.2.3 Generation of paternal half-sib families

Following the summer treatment, seeds from each population were germinated under conditions that mimicked the environment they experience during the autumn

germination period in the field. Seeds from all maternal families were planted in in 2.5 cm x 12 cm Ray Leach Cone-tainers (Stuewe & Sons, Inc.) containing a soil mixture of equal parts of Sunshine Redi-Earth Plug & Seedling Mix (Sungro Horticulture Canada Ltd), silica sand and greenhouse soil, that were placed into water-filled tubs so that the entire soil column was saturated. Tubs were placed into a growth chamber that simulated average winter temperatures and photoperiod of the Central Valley (i.e., 15°C day x 10h and 5°C night per 14h). After germinating, individual seedlings were transferred into a greenhouse with temperature and light cycles that were similar to the spring temperatures in California's Central Valley (i.e., 20 – 25°C day x 14h and 8 – 15°C x 10h; Figure 3.1C), and placed in standing water covering 6 cm of the cone-tainers to simulate spring hydrological conditions in vernal pools. Seedlings were organized into family groups that would be used to generate paternal half-sib crosses by randomly assigning seedlings into groups of four individuals within each population, and then randomly each individual within a group to serve as one of three dams or the single sire (Figure 3.2A). Heterogeneous germination rates led to unequal numbers of groups per population, for a total of 43, 41, and 35 family groups from Dales Lake, Mather Field, and Pixley, respectively. As plants flowered, I conducted hand pollinations between the sire and each dam within each family group to generate an F1 generation that consisted of full sibs (seeds with the same sire and dam) and paternal half-sibs (seeds with the same sire but different dams; Figure 3.2A). Crosses were performed by rubbing a unique sire inflorescence with dehiscent pollen against a single dam inflorescence when its stigmas were exerted (5 – 10 pollinations/infructescence), then discarding the sire inflorescence. Seeds were collected as they became mature. By continuously bottom-watering plants for

several months, I maintained the parental generation in the flowering stage for approximately four months, which allowed individual plants to produce between 4 – 20 inflorescences/plant. This made it possible to generate a large F1 seed population for the common garden experiment that followed.

3.2.4 Greenhouse experiment, planting design and fitness estimation

The paternal-half sib (PHS) crossing design is a standard quantitative genetic approach that allows the additive genetic component of variance to be separated from other sources of variance observed in a population. In my experiment, the additive genetic variance (V_A) component of fitness, which represents the evolutionary potential of a population, was inferred from variation among seeds with different sires (hereafter among-family variance) when growing under one of three different precipitation treatments. Three F1 offspring seeds from each dam (full sibs) were randomly assigned to each of three flooding treatments that were designed to reflect the hydrological patterns that would occur in an extremely dry, average, and extremely wet growing season (Figure 3.2B).

Nine sets of seeds from each full-sib (FS) family were germinated in the growth chamber using the same conditions that were described for the parental generation. Five FS seeds from each maternal plant were planted into nine different cone-tainers with same soil mixture described above for the parental generation. After germination, only one seedling was left per cone-tainer, transferred to the greenhouse, and randomly

assigned to a designated hydroperiod treatment (described below) so that 3 FS (3 seeds from each dam) nested within 3 PHS (seeds from 3 dams with the same sire) were represented in each treatment (Figure 3.2B).

Once the experimental plants began flowering, I performed hand pollinations so that seed production could be used to estimate fitness (Emery et al. 2009, Emery and Ackerly 2014). Throughout the experiment, infructescences were harvested when seeds were visibly mature and nearing abscission. At the end of the experiment, all remaining infructescences were harvested, regardless of their developmental stage. Seeds from an infructescence were stored in coin envelope and placed in a drying oven at 65°C for two weeks, after which they were weighed to the nearest 0.001 mg using a microbalance (Mettler Toledo XP6, Greifensee, Switzerland). I used total infructescence weight as a proxy for fitness because field studies have found that *L. fremontii* infructescence weight is significantly correlated with the total number of viable seeds produced by an individual (Emery 2009). I confirmed that this was an appropriate estimate for the greenhouse-grown plants in this experiment by testing for a statistical association between inflorescence weight and the number of viable seeds that were counted for a random subset of the plants in my study (N= 52 – 70 per population). Consistent with Emery (2009), this analysis found a highly statistically significant relationship between total infructescence weight and the number of seeds produced, so I proceeded to use total infructescence weight as an estimate of viable seed production (and thus fitness) for all remaining statistical analyses. Plants that did not produce flowers were assigned a fitness value of zero (Table 3.2, Figure 3.3).

3.2.5 Hydroperiod treatment design

My experimental flooding regimes were designed to span the range of precipitation regimes experienced by *L. fremontii* populations across its geographic distribution, including both typical and extreme events, such as El Niño Southern Oscillation event (ENSO). To do so, I estimated the hydroperiods at three geographic locations: (1) in the northernmost and wettest population, Dales, during the most extreme El Niño years (extreme high levels of precipitation), (2) at the center population, Mather, during years with no ENSO events (hereafter referred to as "normal" years), and (3) at the southernmost and driest population, Pixley, during the most extreme La Niña years (extreme low levels of precipitation.)

An estimate of the hydroperiod that would be associated with each precipitation regime was calculated by approximating the expected water balance in a typical vernal pool depression at each field site based on historical precipitation data from the past three decades (Table C.1). I used the National Oceanic and Atmospheric Administration (NOAA) Multivariate ENSO Index (MEI) to identify the three years in this dataset that had the highest and lowest MEI indices during the winter months in California, which is indicative of strong El Niño and La Niña events (Wolter and Timlin 1998, Wolter and Timlin 2011). Three intermediate MEI years where ENSO conditions were not reported were randomly selected to represent "normal" years (Wolter and Timlin 1998; Table C.1). I used the California Irrigation Management Information System (CIMIS) from stations closest to each experimental vernal pool complexes to obtain records of daily precipitation and reference evapotranspiration (ET_o) from a standardized grass surface. The site-specific hydroperiod was estimated by comparing the cumulative daily

precipitation (P), cumulative reference evapotranspiration (ET_o) and the cumulative differential between precipitation and evapotranspiration ($P - ET_o$) per each of these three years (McCarten *in review*; Figure 3.4A). I estimated the expected hydroperiod as the total time when the cumulative precipitation exceeded cumulative evapotranspiration for a given growing season (Figure 3.4A). Finally, I calculated the mean hydroperiod across the three ENSO events I had identified in the climate record for each vernal pool complex (Figure 3.4B). These hydroperiod estimates were used to determine the three experimental flooding treatments: (1) the "extreme El Niño" treatment used the average flooding time of the El Niño years at Dales (201 days), (2) the "Normal" treatment used the average flooding time of the normal years at Mather (134 days), and (3) the "extreme La Niña" treatment used the average flooding time of the La Niña years at Pixley (3 days) [Table 3.3, Figure 3.4B].

The three different hydroperiod treatments were imposed by placing the experimental plants in deep irrigation trays (Medium flow trays, Stuewe & Sons, Inc.) and manipulating the length of time that the soil surface was submerged. The cone-tainers containing the experimental seedlings were organized into racks that were placed inside the irrigation trays (Figure 3.2C). Thus, each irrigation tray contained 100 cone-tainers, and there were a total of 11 irrigation trays assigned to each of hydroperiod treatment, for a total of 33 trays and 3,300 experimental plants (3 populations, 35 – 42 PHS families/population). The irrigation trays were randomly assigned to bench positions within the greenhouse, but could not be re-randomized during the experiment due to the weight of the bins when they were full of water (Figure 3.2C). The hydroperiod treatment was imposed by raising the water table 7 cm above the top of the cone-tainers (i.e., soil

surface) for 201, 134, or 3 days to represent the extreme El Niño, normal, or extreme La Niña treatments, respectively. At the conclusion of each treatment, the water table was gradually lowered to simulate the transition from flooded to waterlogged conditions and then from waterlogged to dry (spring maturation period; Table 3.3).

3.2.6 Statistical analysis

Population Responses to Hydroperiod Treatments

I used a mixed model ANOVA to test if populations differed in their fitness responses to the three hydroperiod treatments. Total infructescence weight, which served as my estimate of absolute fitness, was the response variable in this analysis and was log(x + 0.1) transformed prior to analysis to meet the assumptions of ANOVA. Population and hydroperiod treatments were treated as categorical fixed effects, and the population x hydroperiod interaction was included to test if the effects of the hydroperiod treatment on absolute fitness varied among populations. The sire ID of each experimental plant was included as a random effect, and dam was nested within sire to account for the genetic structure embedded in the experimental design.

I used a similar model to evaluate the rank order of populations within each experimental treatment. To focus on the relative performance of populations *within* each treatment, I used the relative fitness of each experimental plant (rather than absolute fitness) as the response variable in the same mixed-model ANOVA described above. Relative fitness was calculated for each experimental plant by dividing its total infructescence weight by the mean infructescence weight of all experimental plants within the same treatment; this variable was also log(x + 0.1) transformed prior to further analysis to meet the assumptions of ANOVA. Upon observing a significant population x

hydroperiod treatment interaction term in this analysis, I conducted post-hoc comparisons among populations within each treatment to identify the specific pairwise comparisons that were statistically different from one another. I used the adjusted Bonferroni corrections for both absolute (total of eighteen comparisons) and relative fitness (total of nine comparisons).

Population- and environment-dependent evolutionary potential

I evaluated the evolutionary potential of populations, and the extent to which this potential depended on the hydrological conditions associated with different precipitation patterns, by conducting log-likelihood tests of mixed-models with different variance structures (Shaw 1991). First, I imposed a model that assumed equal additive genetic variance in fitness among all population and treatment combinations (variance constrained model). I then ran the same model but with a difference variance structure in which the sire variance component was allowed to vary among populations by specifying an unconstrained covariance matrix for the sire x population interaction term. I tested which model best fit the data using a log-likelihood ratio test (Shaw 1991, Saxton 2004). The same approach was used to test if the expression of genetic variation in fitness varied with the experimental treatment (hydroperiod). In this analysis, a model that allowed the sire variance component to vary among hydroperiod treatments was compared to the variance-constrained model. I calculated the best linear unbiased predictor (BLUPs) for each PHS family from these models to estimate the predicted values of the random sire effects (Piepho et al. 2008) to visualize the results. All of the analyses were conducted using the PROC MIX procedure of the SAS System for Unix version 9.4 (Copyright© 2002-2010 by SAS Institute Inc., Cary, NC, USA).

3.3 Results

3.3.1 Population variation in treatment responses

I observed significant differences in plant performance among the three different treatments, as well as average differences among the three populations (Tables 3.4 and 3.5). All three populations had highest fitness under the Normal and extreme El Niño treatments and lowest fitness under the extreme La Niña treatment, generating an overall significant effect of the hydroperiod treatment (Figure 3.5A, Table 3.5). On average, the Mather Field population had the lowest absolute fitness, while the Dales Lake and Pixley Preserve had populations with similar mean absolute fitness across all treatments (Figure 3.5A). However, a significant population x hydroperiod treatment interaction indicated that populations varied in their specific responses to each treatment (Table 3.5, Figure 3.5A), which is reflected in changing rank orders of populations in both absolute fitness (Figure 3.5A) and relative fitness (Figure 3.5B) among the different treatments. Specifically, the Dales Lake population had the highest relative fitness of all three populations under the extreme El Niño and the Normal hydroperiod treatments, but the Pixley population had the highest relative fitness in the extreme La Niña treatment (Figure 3.5B).

3.3.2 Evolutionary potential in response to hydroperiod treatments

An overall significant sire variance component indicated that there was significant additive genetic variance in fitness (i.e., evolutionary potential) among the *L. fremontii* plants included in my experiment (Table 3.5). The heterogeneous variance model comparisons revealed that the amount of additive genetic variation did not significantly

vary among the three populations (variance constrained model vs. heterogeneous sire variance among populations: X^2 = 5.5 DF= 5.0, P < 0.36). However, the expression of this additive genetic variance in fitness did differ among the hydroperiod treatments, as reflected by a significant interaction between the sire variance component and treatment Table 3.5, Figure 3.5), and an improved fit of a model that allowed the sire variance to differ among treatments (compared to variance constrained model: X^2 = 30.7, DF= 5.0, P < 0.01). The evolutionary potential was greatest in the extreme La Niña treatment (sire variance = 0.4373), where the sire variance component was almost three times greater than the extreme El Niño (sire variance = 0.1269) and twenty times greater than the Normal (sire variance = 0.02389) treatments (Figure 3.5).

3.4 Discussion

As a vernal pool endemic plant species, *L. fremontii* has a very limited capacity to track climate change via dispersal due to the patchy distribution of its habitat and its limited potential for long-distance seed dispersal. Thus, the persistence of this species will heavily depend on its adaptive capacity to evolve *in situ* in response to changing climatic conditions. My experiment evaluated the effects of extreme precipitation events (as mediated through the hydroperiod) on the mean fitness and evolutionary potential of *L. fremontii*. I found that *L. fremontii* populations varied in their responses to extended or abbreviated hydroperiods, but exhibited similar overall levels of additive genetic variation in fitness. Populations expressed the highest adaptive potential (additive genetic variation in fitness) under conditions that simulated the hydroperiod under a particularly dry La Niña treatment, which was also the treatment in which populations showed the lowest mean absolute fitness. Thus, populations expressed higher V_A in fitness under treatment that was the most stressful for the species.

Latitudinal differences in precipitation across California (Figure 3.1A) may have driven divergence in climatic tolerances among populations across the species range. The northernmost and southernmost populations each outperformed the other populations under the extreme conditions that have historically occurred in their home locations: Dales Lake showed the highest relative fitness in the El Niño treatment (Figure 3.5B), while Pixley had the highest relative fitness in the La Niña treatment (Figure 3.5B). The population from Mather Field had the lowest fitness in all the treatments (Figure 3.5). These results are consistent with the hypothesis that selection has favored tolerance to relatively dry conditions at the southern edge of the species range, and tolerance of

extended flooding at the northern range edge. This could be a result of California's latitudinal location, which generates opposing changes in precipitation along the state. Southern areas are usually sub-tropical and relatively dry, while the northern regions are relatively wet (Berg and Hall 2015, Cayan et al. 2008). As a result, northern California receives greater annual rainfall than the southern region during El Niño events, and southern California is disproportionately warmer and drier during La Niña conditions (Cayan et al. 1999, Wang and Kumar 2015). Thus, the PVP and DL populations may be more adapted to relatively short and long periods, respectively, due to the precipitation patterns that have historically characterized their locations. While a formal test of local adaptation would require reciprocal transplants among all sites across a range of precipitation conditions, including an El Niño – La Niña cycle, the results of my experiment are consistent with the hypothesis that populations at the range edge are locally adapted to the climatic extremes that they have experienced in recent history.

While the rank order of relative fitness provides insights into possible patterns of local adaptation in *L. fremontii* (Blanquart et al. 2013, Kawecki and Ebert 2004), the mean absolute fitness of each population under the alternative treatments illustrates the extent to which populations experience stress under the different precipitation regimes. I discovered that the populations from the edge of the species' geographic range exhibit more robust phenotypes overall than the population from the range center (MF). Populations from the northern and southern ranges had higher absolute fitness than the central population under extremely wet or dry conditions, respectively, and the northern population outperformed the central population even in the Normal treatment. Thus, the two marginal populations exhibited relatively broad climate tolerance (i.e., greater

ecological amplitude) which may allow them to maintain relatively high mean fitness under each extreme scenario. However, the central population from Mather Field showed relatively low mean fitness under all treatments, suggesting that this population has overall lower fitness than the marginal populations, regardless of the precipitation regime (as in Halbritter et al. 2015). The populations from DL and PVP may be more tolerant to extreme rainfall events because those conditions have historically defined their local precipitation regimes in comparison with Mather Field, whose central position might have historically exposed this population to less extreme precipitation patterns during ENSO events (Figure 3.4B). Fluctuating selection—as likely occurs in the vernal pool habitats—can favor plasticity in traits that maintain population fitness across a broader range of environments (Dejong 1995, Levins 1968). Higher plasticity in peripheral compared to central populations may explain the broad ecological tolerance of DL and PVP. Thus, further research that compares the plasticity of traits within these populations and across the treatments will make it possible to link trait variation to plant fitness in the different environments (Torres-Martínez et al. *in preparation*).

The geographic isolation and unique histories of drift and gene flow that characterize *L. fremontii* populations (Torres-Martínez and Emery *in press*) have not created differences in the additive genetic variance in fitness within these populations, even in response to extreme climate events. Populations did not vary in the amount of additive genetic variance that they expressed, averaged across all treatments (Table 3.5). In contrast to my results, other studies that have tested for inter-population differences in response to future climate conditions (using latitudinal gradients as a proxy of these projected environments) have documented differences in the V_A of fitness-related traits

among populations. For instance, Etterson (2004) reported higher expressed V_A in southern populations from historically drier sites compared to northern population from relatively wet sites when they were reciprocally transplanted across locations. Similar results have been observed in populations of invasive species under conditions in which they are invasive (e.g., Matesanz et al. 2014). One possible explanation for my results is that the variable environment of vernal pools and self-incompatible mating system of *L. fremontii* have maintained similarly high levels of genetic variation within each population, even in marginal populations at the edges of the species range where I might expect genetic variation to be relatively low (Provan and Maggs 2012, Sexton et al. 2009).

A particularly important result from my work is that expression of additive genetic variation in fitness changed with the climate experienced by the populations (Figure 3.6). In fact, the highest additive genetic variation in fitness was expressed in all populations under the most stressful environment (as defined by the treatment in which population mean fitness was lowest)—the extreme La Niña. Previous studies have reported that the expression of higher additive genetic variation of populations under stressful or novel conditions can be due to their past history of selection because past selection pressures (either directional or stabilizing) have maintained the expression of the same gene combinations, but once those pressures change (e.g., novel environmental conditions) new gene combinations can be expressed and increase the overall genotypic and phenotypic variance (Hoffmann and Merila 1999, Hoffmann and Parsons 1991, Imasheva et al. 1998, Schlichting 2008, Sgro and Hoffmann 1998). Similarly, (Zhivotovsky et al. 1996) observed that rare and poor environments can cause mean

population fitness to decrease but the genotypic variance to increase. Recently, Shaw and Shaw (2014) demonstrated through simulations that abrupt changes in selective pressures between generations can cause an increase in $V_A(W)$ but a reduction in mean population fitness. On the other hand, consistent patterns of selection will enable populations to persist for many generations, even if they exhibit low adaptive capacity. An important aspect of the evolutionary history of *L. fremontii* is that this species has evolved from terrestrial ancestors, and thus may harbor genetic variation for terrestrial-like conditions that is not expressed under the typical flooded conditions (Emery and Ackerly 2014).

Taken together, my results suggest that *L. fremontii* populations may have different mechanisms for responding to the opposing climatic extremes (high vs. low precipitation) that are projected to become increasingly common in California over the next century (e.g., Yoon et al. 2015). In years with unusually high levels of precipitation, as has occurred in the past during extreme El Niño events, populations exhibit low evolutionary potential (low $V_A(W)$; Figure 3.6), but high mean populations fitness ($\bar{W}$; Figure 3.5A). Thus, *L. fremontii* populations will likely persist under these precipitation regimes because these conditions are not particularly stressful for the plants. In contrast, during particularly dry growing seasons, such as past extreme La Niña events, populations showed low mean absolute fitness ($\bar{W}$; Figure 3.5A) but high evolutionary potential ($V_A(W)$; Figure 3.6). Thus, these conditions may cause population size to be reduced, but will favor a rapid adaptive response (Fisher 1930, Shaw and Shaw 2014). However, it is important to take into account that this evolutionary potential may not persist under permanent and repetitive drought events. Previous studies of seed germination in these *L. fremontii* populations have found relatively low seed viability in

the southernmost populations in comparison with the central and northernmost populations, which could also challenge population persistence at the southern range of the species. Furthermore, a field experiment conducted by Emery and Ackerly (2014) found that genetic variation in the hydrological response curve of *L. fremontii* is only expressed when competitors were removed. Thus, the patterns of genetic variation in fitness observed here could be reduced if the experimental plants were competing with other vernal pool vegetation in their native environment.

In conclusion, *L. fremontii*'s species-wide response to extreme climate events will depend on the individual responses of its constituent populations. My results indicate that populations from different positions within the species' range, and thus that have experienced different recent evolutionary histories in their respective environments, will exhibit distinct demographic and evolutionary responses to extreme climatic events. The effects of climatic extremes on population mean fitness can to some extent be predicted by the local historical climate of each population. Overall, range edge populations appear to be more robust than the central population in my study, and thus the marginal populations might not be as vulnerable to climate change as current predictions would suggest (Anderson et al. 2012, Provan and Maggs 2012, Razgour et al. 2013, Sexton et al. 2009), but will likely play an important role in the species persistence (Rehm et al. 2015). Collectively, my results highlight the importance of evaluating the population- and environment-specific patterns of additive genetic variation in fitness to predict species responses to increasingly variable climatic regimes.

Table 3.1 Geographic locations and ownership information of the three vernal pool complexes from which *L. fremontii* seeds and climate data were collected.

Location Name	County	Property Owner	Latitude (N)	Longitude (W)
Dales Lake Ecological Reserve	Tehama	CDFW	40.32878	-122.06222
Mather Field	Sacramento	Sacramento County DP&R	38.53748	-121.26267
Pixley Vernal Pool Preserve	Tulare	Center for Natural Lands Management	35.98547	-119.21205

Table 3.2 Results from an ANCOVA that tested if infructescence weight can be used to predict the number of viable seeds per individual. The significant relationship justified the use of infructescence weight as an estimate of fitness.

Source	DF	MS	F	P
Infructescence weight	1	83912.9	168.6	<0.01
Population	2	41.46	0.08	0.92
Infructescence weight*Population	2	114.45	0.23	0.79

Table 3.3 Hydroperiod treatments that were imposed to simulate local and extreme annual precipitation levels in the greenhouse experiment. The conditions at the beginning (autumn germination conditions) and end (early summer conditions) of the life cycle were standardized across all treatments (see Methods).

Treatment	Autumn Germination Period (Standardized)	Winter Submergence (Hydroperiod Treatment)	Spring Maturation Period (Standardized)*			
			Initial Drop in Water	Saturation	Moist	Total Drainage
Extreme El Niño	4 weeks	201 days	10 days	2 weeks	2 weeks	~ 1 week
Normal	4 weeks	134 days	10 days	2 weeks	2 weeks	~ 1 week
Extreme La Niña	4 weeks	3 days	10 days	2 weeks	2 weeks	~ 1 week

*Within each irrigation tray we drained the water at a rate of 1 cm per day for 10 days, or until the total water level reached 6 cm below the soil surface (***initial drop in water***). We maintained these waterlogged conditions for two weeks (***saturation stage***) and then drained 5 cm more from each irrigation tray, so that the water covered only 1 cm of the bottom of the cone-tainers (***moist stage***). This water level was maintained for another two weeks, at which point it was allowed to evaporate naturally (***total drainage***).

Table 3.4 The mean absolute and relative fitness of each population in each hydroperiod treatment.

Population	Hydroperiod Treatment	Absolute Fitness		Relative Fitness	
		Mean	SE	Mean	SE
Dales Lake	Extreme El Niño	10.55	0.34	1.21	0.04
Dales Lake	Normal	10.51	0.27	1.2	0.03
Dales Lake	Extreme La Niña	3.12	0.22	0.99	0.07
Mather Field	Extreme El Niño	6.91	0.26	0.79	0.03
Mather Field	Normal	7.53	0.25	0.86	0.03
Mather Field	Extreme La Niña	1.54	0.16	0.49	0.05
Pixley	Extreme El Niño	8.54	0.42	0.98	0.05
Pixley	Normal	7.93	0.34	0.91	0.04
Pixley	Extreme La Niña	5.17	0.25	1.64	0.08

Table 3.5 Results from a mixed model nested ANOVA that tested the effects of Population and Hydroperiod Treatment on the absolute fitness among populations under flooding treatments. Sire nested with Population, Dam nested within Sire, and the Treatment x Sire interaction were included as random effects in the model to account for the quantitative genetic design of the experiment.

Effect	DF	MS	F	P
Population	2	138.29	65.52	< 0.01
Hydroperiod Treatment	2	1383.87	655.69	< 0.01
Population* Hydroperiod Treatment	4	18.41	56.1	< 0.01
Covariance Parameters	**Estimate**	**Error**	**Value**	**P > Z**
Sire (Population)	0.08	0.03	2.42	< 0.01
Dam (Population*Sire)	0.08	0.03	2.61	< 0.01
Treatment*Sire (Population)	0.09	0.03	3.11	< 0.01
Residual	1.87	0.05	34.21	< 0.01

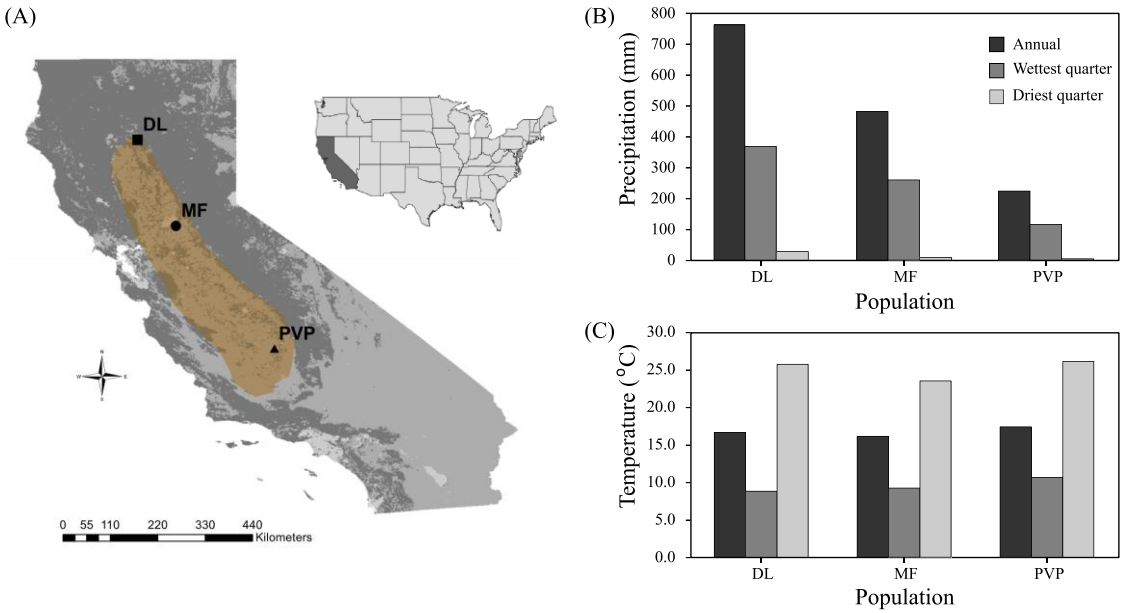

Figure 3.1 (A) The geographic distribution *Lasthenia fremontii* in the Great Central Valley of California (orange) and the three vernal pool complexes that are the focal populations and sites in this study: Dales Lake (DL) from the northernmost range edge, Mather Field (MF) from the center of the species range, and Pixley Vernal Preserve (PVP) from the southern range edge. Climate data from 1950-2000 that were extracted from the Worldclim database demonstrate that the three focal populations originate from sites that span a latitudinal gradient in (B) total annual precipitation and the precipitation of the wettest and driest quarters. However, these sites have not experienced markedly different patterns in (C) annual mean temperature or the mean temperature of wettest and driest quarters over the past several decades.

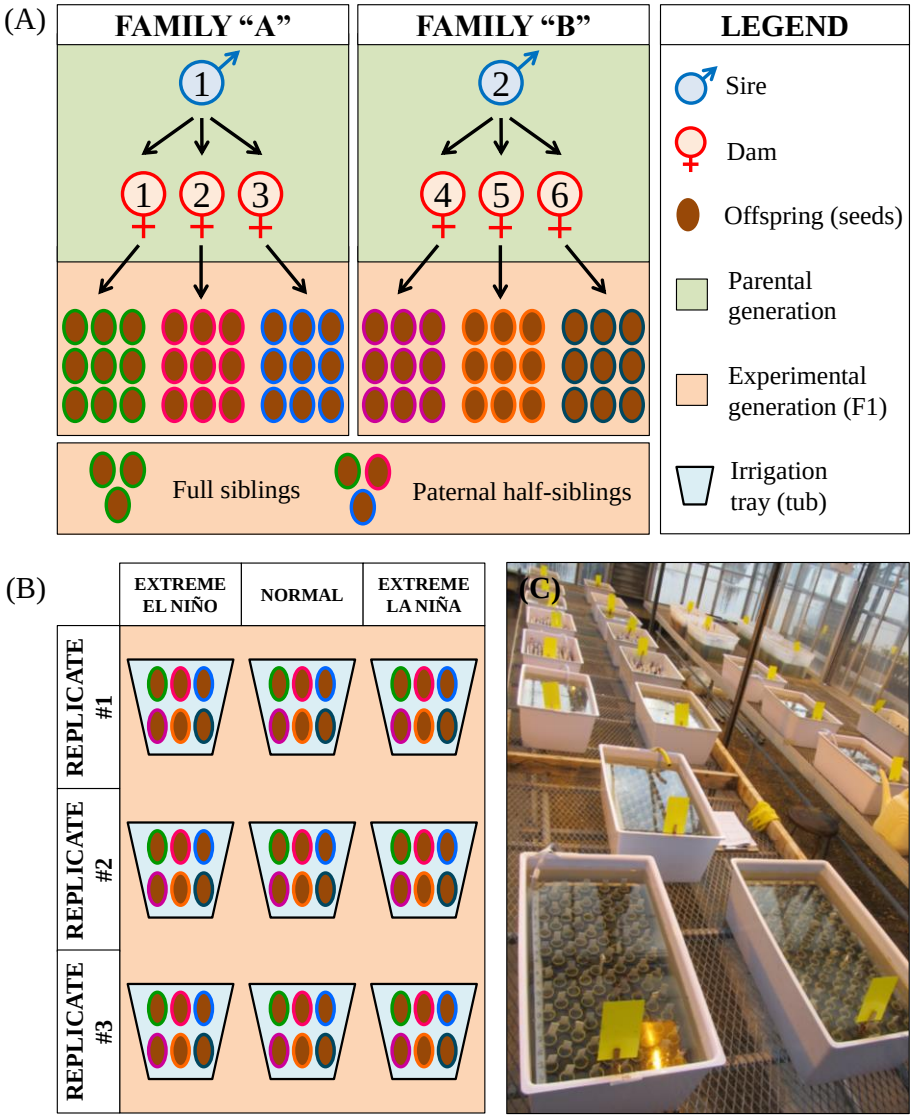

Figure 3.2 Paternal half-sib crossing design and experimental treatments for evaluating the expression of additive genetic variation in fitness, $V_A(W)$, under different climatic conditions. (A) Sires and dams were sorted into families (parental generation). Within each family, the F1 generation consisted of paternal half-sibs (same dam) and full-sibs (same dam and sire) nested within the paternal-half sibs (PHS). (B) Three F1 offspring per dam were randomly assigned to each flooding treatment to have 3 full-sibs per dam, nested within a PHS family with 3 unique dams, per experimental treatment.
(C) Photograph of the experiment under way in the greenhouse.

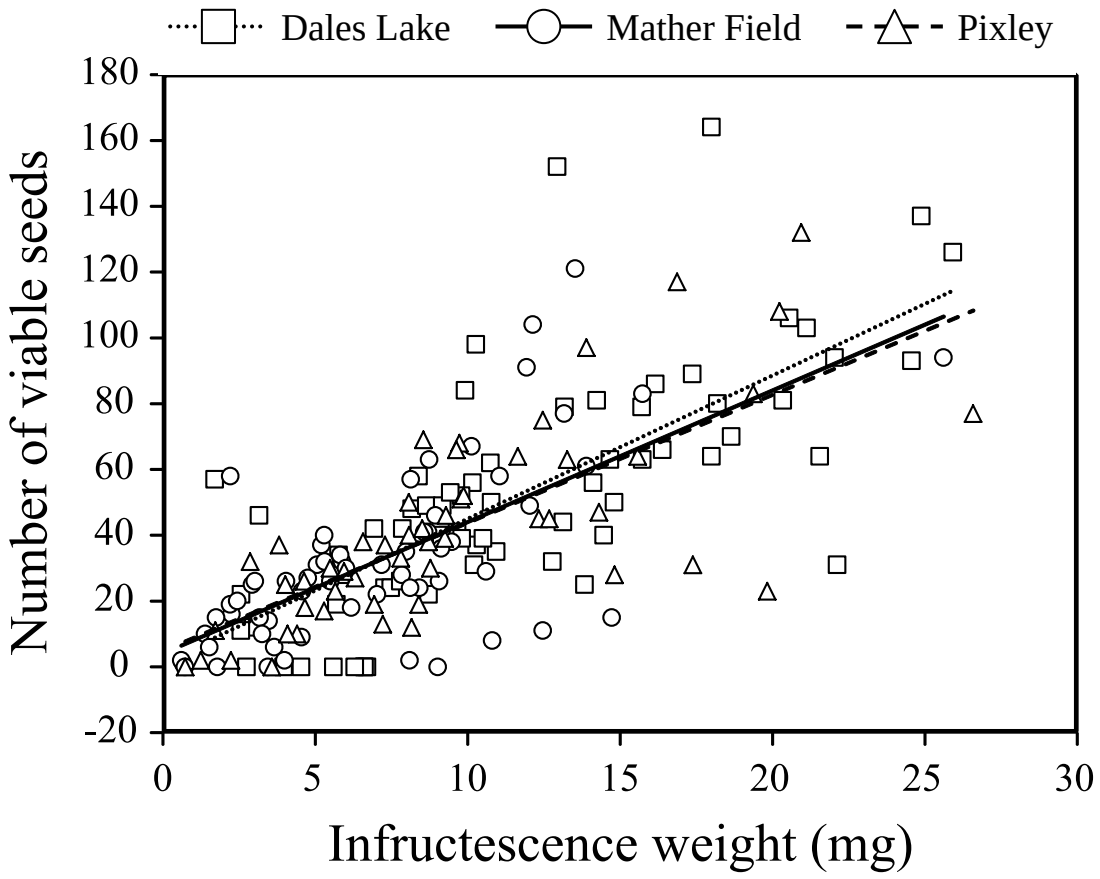

Figure 3.3 The number of viable seeds is significantly correlated with the total infructescence weight of a plant (one-way ANCOVA with Population as a main effect and infructescence weight as a covariate; overall model $P < 0.01$, covariate effect $P < 0.01$). This relationship was consistent across the three populations (Population effect: $P = 0.92$).

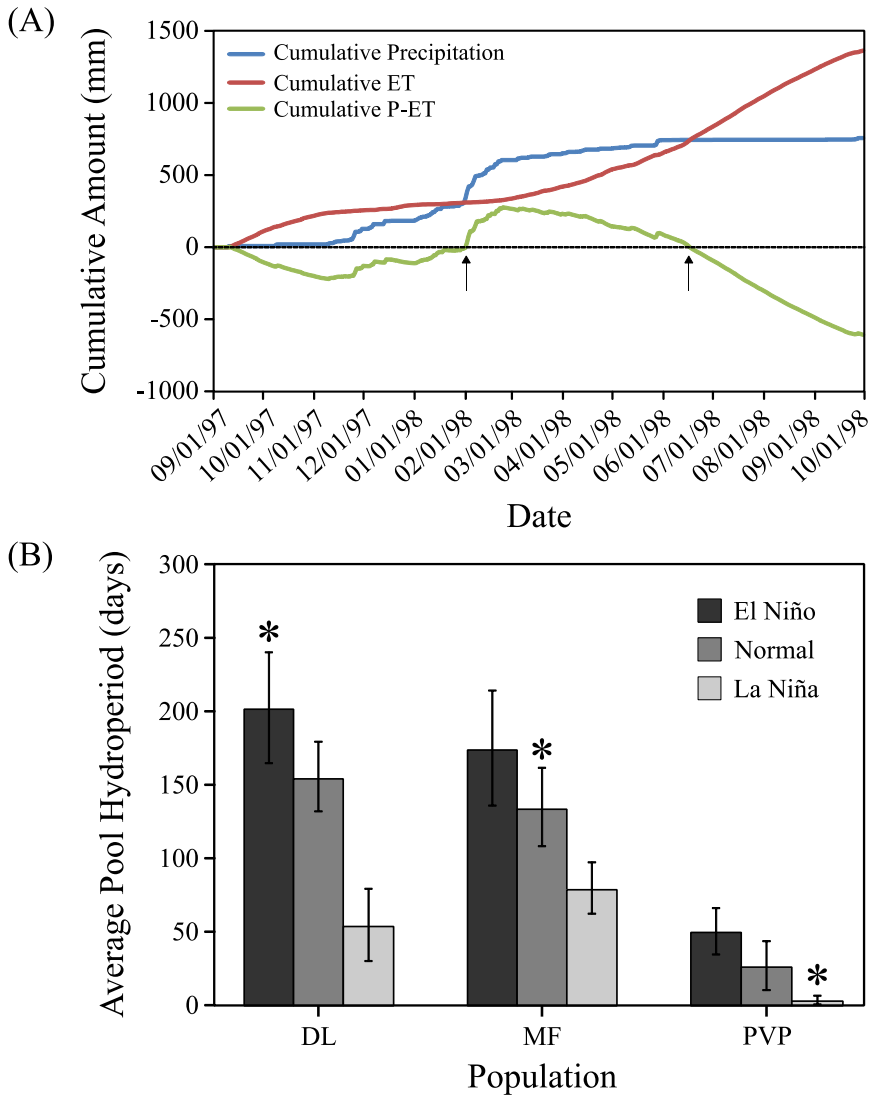

Figure 3.4 (A) An example of how the water balance was calculated and used to design experimental hydroperiods. The data from only one site and year are shown here for illustrative purposes only. The two arrows bracket the time period during which a pool was considered to be flooded because the cumulative daily precipitation (P) exceeds water loss due to the cumulative evapotranspiration (ETo) at that site. (B) The mean hydroperiods estimated for pools at each of the three field sites under three different precipitation scenarios, as estimated using the three most extreme ENSO events and three "normal" years from historical data spanning the last three decades. The experimental treatments were selected to represent the most extreme wet and dry conditions, as well as conditions representing "normal" precipitation patterns.

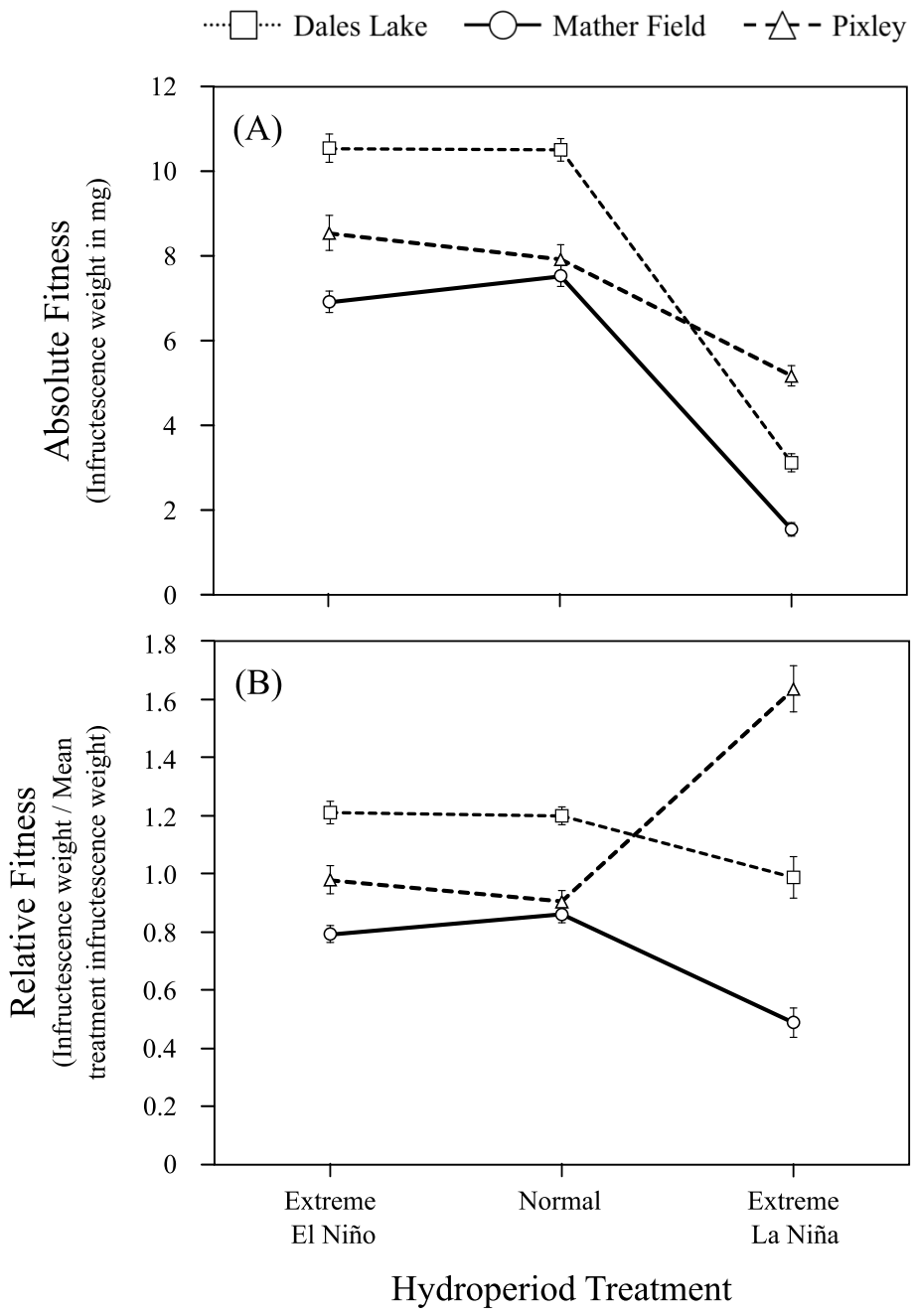

Figure 3.5 Fitness of populations within and across experimental hydroperiod treatments. Points represent (A) the mean absolute fitness (infructescence weight) and (B) mean relative fitness (individual infructescence weight / mean infructescence weight in treatment). Error bars represent ± 1 standard error. Populations from Dales Lake and Pixley showed evidence of local adaptation because they had higher fitness than other populations under the extreme conditions that were estimated from their locations. The Mather Field population had lowest absolute and relative mean fitness under most conditions.

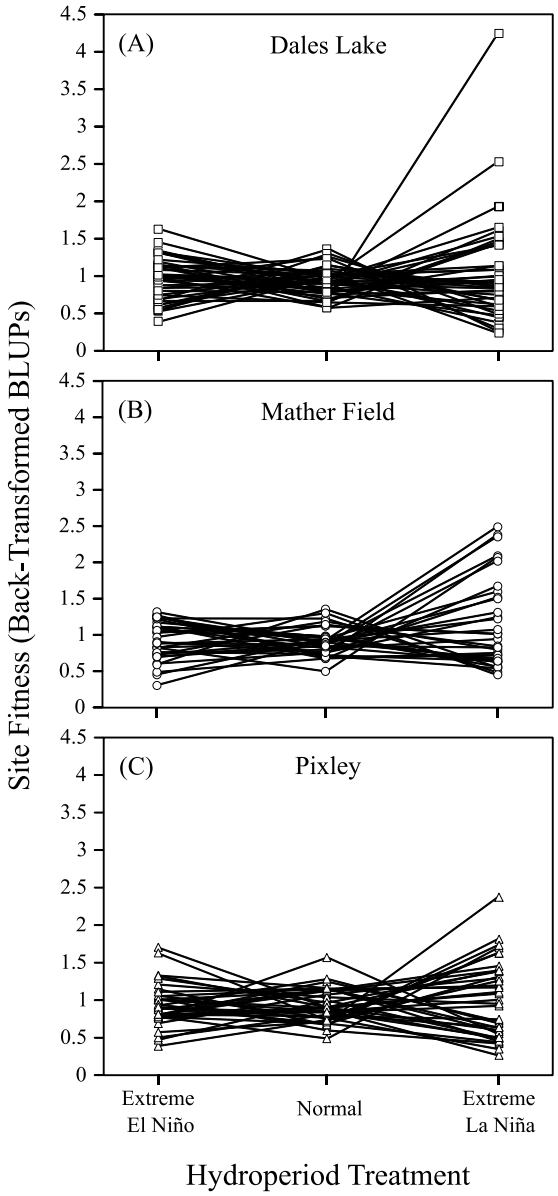

Figure 3.6 Response curves of each PHS family across each experimental treatment. Each line represents the mean fitness of seeds that share the same sire but different dams, estimated using BLUPs. Data are presented separately for each population (A) Dales Lake, (B) Mather Field, (C) Pixley. The dispersion of the points within a given treatment indicates among-sire variance.

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APPENDICES

Table A.1. The barcode ID and number of reads obtained for the twelve *L. fremontii* individuals in the RADseq library, before and after trimming each read to 75 bp and filtering with Stacks. The RADseq library for SNP discovery contained one individual from each of the 12 different locations (Figure 1). Location abbreviations correspond to those in Figure 1.1 and Table 1.1.

Location (abbreviation)	Barcode	Number of Reads		
		Raw	Post-trimming	Post-filtering
Dales Lake Ecological Reserve (DL)	GCGACC	5,316,302	5,253,257	5,250,077
Thomes Creek Ecological Reserve (TC)	TCAAAG	2,299,743	2,275,468	2,274,237
North Table Mountain Ecological Reserve (NTM)	TCCTGC	2,502,585	2,480,535	2,479,242
Vina Plains Preserve (VINA)	AGTTAA	3,192,444	3,150,627	3,147,857
Beale Air Force (BAF)	TTGAGC	3,987,826	3,923,826	3,919,534
Mather Field (MF)	GCATGG	2,433,639	2,411,778	2,410,635
Glide Tule Ranch (GT)	GCGCTG	1,866,077	1,847,454	1,846,669
Jepson Prairie Preserve (JP)	GCCGTA	2,644,757	2,621,792	2,620,750
Arena Plains (ARE)	GGGGCG	2,075,330	2,052,693	2,051,322
Big Table Mountain Ecological Reserve (BTM)	ATGCAC	2,230,023	2,205,006	2,203,341
Pixley Vernal Pool Preserve (PVP)	CTTATG	1,089,327	1,075,594	1,074,973
Carrizo Plains National Monument (CARR)	ATCAAA	2,839,573	2,807,086	2,805,404
Total		32,477,626	32,105,116	32,084,041
Average		2,706,469	2,675,426	2,673,670

Table A.2 Number of RAD loci obtained using different parameter settings in STACKS for *de novo* SNP discovery.

	Catalog ID									
	1	**2**	**3**	**7**	**5**	**6**	**8**	**9**	**10**	**11**
Parameter Settings										
m	4	4	4	10	10	10	10	10	10	10
M	2	2	1	2	2	2	2	3	4	5
n	2	3	1	2	3	4	5	2	2	2
Summary of Sequence Output										
# Loci in catalog	434716	420543	463606	316106	292852	285619	279990	298861	296288	294248
# Loci with SNPs	33050	36068	23838	21785	27983	29491	30555	27983	28275	28903
# Loci present in ≥10 samples	1386	1722	862	754	1451	1631	1743	1452	1174	1175
# Loci with SNPs present in ≥10 samples	1350	1687	817	713	1415	1594	1707	1415	1137	1141
# Monomorphic loci in ≥10 samples	36	35	45	41	36	37	36	37	37	34
% Monomorphic loci in ≥10 samples	2.6	2.03	5.22	5.44	2.48	2.27	2.07	2.55	3.15	2.89
# Polymorphic loci in ≥10 samples	1350	1687	817	713	1415	1594	1707	1415	1137	1141
% Polymorphic loci in ≥10 sample	97.4	97.97	94.78	94.56	97.52	97.73	97.93	97.45	96.85	97.11
# Loci with 2 SNPs in ≥10 samples	82	74	134	78	75	64	61	75	72	71
# SNP candidates for genotyping in ≥10 samples	132	121	194	135	128	116	112	128	124	120
# SNP candidates for genotyping in ≥2 samples	15289	12510	13361	10142	10142	7535	6957	9411	8932	8557

Table A.3 Sequence information for 71 SNPs that were validated and genotyped in 285 *L. fremontii* individuals that were collected from 10 populations that collectively spanned the species' geographic range. The ID of the catalog locus from which each SNP was discovered (first column), the unique ID for each SNP (second column), and the position of the SNP in the consensus sequence (third column) are provided. I report the primer sequences for genotyping that were developed using KASP chemistry, which is based on competitive allele-specific PCR chemistry that consists of two competitive allele-specific forward primers (fourth and fifth columns) and one common reverse primer (sixth column). The 44 loci that were polymorphic among complexes are highlighted in bold.

Catalog ID	SNP ID	SNP position	Primer_AlleleFAM	Primer_AlleleHEX	Primer_Common	Alleles FAM/HEX
2413	t7_1	39	CCCACACGCCGCTTGCAC	CCCCACACGCCGCTTGCAT	TGGCTGTAAAATCGCAGCCGAGAAA	C/T
3534	**t7_2**	**49**	**AGAGGAACAAGCATTACAACTTGTT**	**AGAGGAACAAGCATTACAACTTGTC**	**GAGATGCTGGCTTCACAAGAATCCAA**	**A/G**
6746	t7_3	32	CAGGTGAAAAATAATTAAAGCTTATGTCG	GCAGGTGAAAAATAATTAAAGCTTATGTCA	TCTCCTACTCAATGCGATTATGACTTAAAA	G/A
12837	t7_4	31	CAGGGTATGGAAAACTTTTTTCTCATAAC	CAGGGTATGGAAAACTTTTTTCTCATAAT	TTTACTGTTCGAGAAAACATAGGATTATAT	C/T
13591	**t7_5**	**24**	**CAATAATGAGTAATCTAATGCAGGGTGTT**	**ATAATGAGTAATCTAATGCAGGGTGTC**	**TGCAGGTTCCCCCTTCCACGTT**	**A/G**
15536	**t7_7**	**54**	**AGAGAGAAGGTGTGGGCGAATA**	**AGAGAGAAGGTGTGGGCGAATG**	**CAACAACCCATACATCCGCTGTATATTTA**	**T/C**
3475	t7_11	24	GCAGGCATTGTGTAAAACACTGG	GCAGGCATTGTGTAAAACACTGA	ACTTAAAACTTTTGTTAGTTAGAACGCCAA	G/A
10076	**t7_15**	**34**	**TTGGGGATTGTTTTGGCTTTTGGC**	**TTGGGGATTGTTTTGGCTTTTGGT**	**CAATCAGCCACCCGCCAAGCAA**	**C/T**
11118	**t7_16**	**25**	**GCAGGTCCAAGTATAATTAGCTTC**	**GCAGGTCCAAGTATAATTAGCTTT**	**TCGTCCCTTTTGACCAAAGACCTTATTTA**	**C/T**
13629	**t7_17**	**43**	**GGAGCTAGTGAAGGACCCGGA**	**GGAGCTAGTGAAGGACCCGGT**	**CCACCGGTTTTACCTATATACAATGCAAT**	**T/A**
14316	t7_18	47	CCAATAACAGCTGTACAACTGGAGTT	CAATAACAGCTGTACAACTGGAGTG	CTCTTCTTCTGCATCTCTAGTCCACAA	A/C
19416	**t7_19**	**31**	**GCATGTCTAATAGAACTATATTTAAGCAAG**	**CGCATGTCTAATAGAACTATATTTAAGCAAA**	**GCTGCAGCAACACAAACTCTGCATA**	**C/T**
1294	**t7_21**	**27**	**CAGGTATGTATTTGCAAGGCCTTCT**	**AGGTATGTATTTGCAAGGCCTTCC**	**GTTCCGTTTGATGAAACAACAAAGCATCTT**	**T/C**
3293	**t7_22**	**23**	**GCAGGAACTAGTGCTGTACGCA**	**CAGGAACTAGTGCTGTACGCC**	**GAAGAGGACCAGGCAGCTGAGA**	**A/C**
4011	t7_23	44	GTCACTACTGGCTACACAAA	GCTGTCACTACTGGCTACACAAG	ACACTGGAAGCTAGACATCAATTTGGTA	A/G
5388	t7_25	46	TGAATCAGAATAGACCACTTTATACTTTC	ATATTGAATCAGAATAGACCACTTTATACTTTT	GGTCTTGGCACAAAAACAGATGGAT	C/T
8564	**t7_26**	**34**	**TAGCACATATTATAATTAATATGTGATTAAAA**	**AGCACATATTATAATTAATATGTGATTAAAG**	**TGCAGGTATAGTATCATCACTCTTGTTTAA**	**T/C**
10553	**t7_27**	**39**	**CCCGAAACACTACTTTATAGGCAGA**	**CCGAAACACTACTTTATAGGCAGG**	**CCTGTTTGAGCTTTCCCTGTACATAATAA**	**T/C**
14704	**t7_28**	**24**	**GCAGGCTAAAACAAAATCGGCTT**	**GCAGGCTAAAACAAAATCGGCTC**	**CTGAGTACAAATATGTTGGTCCTAATCAAA**	**T/C**
15370	**t7_29**	**38**	**ATCAGGGTAAAGATTCATTGTTCTTGC**	**GAATCAGGGTAAAGATTCATTGTTCTTGT**	**GGCAATGGATCCGACAAAACTTCATTTAT**	**C/T**
15442	t7_32	36	TTATAAAAGATGATGTGTGAAATGATTTGATA	TATAAAAGATGATGTGTGAAATGATTTGATT	GGTCAGATTCCAATATCTTATTTATTCCTT	T/A
1614	**t7_33**	**41**	**TAATGAGCAATATAGAATGAAGTAAAGAAAAA**	**AATGAGCAATATAGAATGAAGTAAAGAAAAG**	**TGCAGGCTAATTATATCATTTATAATCCAT**	**T/C**
2839	**t7_34**	**43**	**ACGGTTTTACCCTTCCGCAATCA**	**CGGTTTTACCCTTCCGCAATCC**	**GGCGTATGGGGGTTGACCGAAA**	**T/G**
3380	t7_35	24	GCAGGTTGTGATGATTGTGAAAT	GCAGGTTGTGATGATTGTGAAAG	AGTTTCTCTGCATTCTCAGTTCTCTCAT	T/G
4716	**t7_36**	**38**	**TTGATTTGCTTTATAATCAACATCAATAAGAC**	**TGATTTGCTTTATAATCAACATCAATAAGAT**	**ATAGATTAGAAATCGAATCAGAGCTCCTTA**	**C/T**
5211	**t7_37**	**43**	**GAAAAAAAACCATTTACTTTTTAACTTTGCTC**	**GGAAAAAAAACCATTTACTTTTTAACTTTGCTT**	**TTGGAAGCTCGGAGTCGTCACAAT**	**G/A**
6829	**t7_38**	**35**	**GGTTTTGATTATTACCATTGTTTGATGTTGT**	**GTTTTGATTATTACCATTGTTTGATGTTGG**	**GAATTTGAAAGCAATCAATTTACATTTAGT**	**T/G**
8887	**t7_39**	**43**	**CCGCAGCTGTTGTTTCACAACCT**	**CGCAGCTGTTGTTTCACAACCC**	**GGTACCCACTGCGGTGGTTGTT**	**A/G**
9290	**t7_40**	**38**	**AGATTAATCTCTTAATCTTAAACGTTTGAATTG**	**AGATTAATCTCTTAATCTTAAACGTTTGAATTA**	**GCAGGTACTTTCAATGCATCTTTGATCTA**	**C/T**

Table A.3 (Continuued)

Catalog ID	SNP ID	SNP position	Primer_AlleleFAM	Primer_AlleleHEX	Primer_Common	Alleles FAM/HEX
10325	**t7_42**	**36**	**ATTCGTCCAAAAAAGAAAAATCAAACGAAATT**	**CGTCCAAAAAAGAAAAATCAAACGAAATC**	**GACGGAAGGAAGGATTCTTCGTTGTT**	**A/G**
11943	t7_43	43	TACATAACTAATAACCACCTCCAACG	ACATAACTAATAACCACCTCCAACC	AGGATCCTCCATGGGTTTTGTTTTCATAT	C/G
13521	**t7_45**	**43**	**GGATGTTGTGGGCTAGAGTGG**	**GTGGATGTTGTGGGCTAGAGTGA**	**TCAGCGGTGACGAGCGGTCAA**	**G/A**
14025	**t7_46**	**30**	**GATCCAGCTAGTGGCTCACGT**	**GATCCAGCTAGTGGCTCACGC**	**TGCAGGCAGCACCTGAGC**	**A/G**
17839	**t7_49**	**40**	**GCCTGCTGATATTAACAAACCTCCAA**	**CCTGCTGATATTAACAAACCTCCAG**	**CGGTTCTAGAAATAATCTTGTAGCATGGAA**	**T/C**
18727	**t7_50**	**39**	**GAAAAGAAGACAAATCTTGTAAATCCCAG**	**GAAAAGAAGACAAATCTTGTAAATCCCAC**	**GCACATGATTTCCCAATGCATGAAAGAAA**	**C/G**
21125	**t7_52**	**46**	**CAGCCGATACATTGAGAATGCCG**	**CAGCCGATACATTGAGAATGCCA**	**AATGAAAGTTCGATTTAGCATAGATGCCAA**	**C/T**
4386	t7_53	41	AATTTAATCCTTTTAGGTTAAATTTTTGTGGC	GAATTTAATCCTTTTAGGTTAAATTTTTGTGGT	GCAGGTCATACACATAACAATAGACGAAA	G/A
11705	**t7_56**	**20**	**GCAGGACGACACATCAGCC**	**GCAGGACGACACATCAGCT**	**GTTCGATGAACTTTTGACAGTGTAGCATA**	**C/T**
12363	t7_57	30	GGTGAACACAAGCATCTGCTACTA	GTGAACACAAGCATCTGCTACTG	GCTCATTTCAATCCAAGCCTTTAAAGCAA	A/G
14614	**t7_59**	**26**	**GCAGGCATAAAGATTCGAACATGAG**	**GCAGGCATAAAGATTCGAACATGAA**	**GACTCTTATCTGTTTGTGCTTATCACACAT**	**G/A**
18796	t7_61	31	GAGTGGTATTTGGTTTGTTACGGATC	GAGTGGTATTTGGTTTGTTACGGATG	TGTTGCGCGAGCAGAGAGCCAA	C/G
2372	t7_65	42	AAAGATGAATGTTTGCTTCAAGATAGATGT	GATGAATGTTTGCTTCAAGATAGATGC	CAACTCAGCCGAAACATACAAAATTCCAT	T/C
8090	**t7_66**	**45**	**CTAGTAAGATTTTTACATGTGGTTACGA**	**CTAGTAAGATTTTTACATGTGGTTACGC**	**ATACAGGTTTCATACGAACCTCTAAGCAA**	**T/G**
8138	**t7_67**	**37**	**CGATGACCGCAACACGAAGGTTA**	**GATGACCGCAACACGAAGGTTG**	**CAGCCCCAAAATCACTGGCCG**	**A/G**
10901	t7_70	23	GCAGGCTGCATCTCAGTTAGTC	GCAGGCTGCATCTCAGTTAGTT	GCAGCCTGCTTCGTATTTTGACATTTTAA	C/T
11429	**t7_71**	**36**	**CAATAAAAAAGCATATTAAGTGGCTATTTAAGT**	**CAATAAAAAAGCATATTAAGTGGCTATTTAAGA**	**CCTGCAATTGCATGACCCTTTATTCTATT**	**A/T**
15349	**t7_75**	**53**	**GATTCTCAAGGGTCAGATCTCGC**	**CTCAAGGGTCAGATCTCGG**	**CTGGAAACGATGATGGTAAGAAGTTTGTA**	**G/C**
15349	t7_76	37	ATTATCAAGAACACAAGAACACACCG	AATATTATCAAGAACACAAGAACACACCA	TGCAGGGTATATGATTCGCAAATCATCAA	C/T
18789	**t7_77**	**41**	**AAGTTTTCTCCCAATGGGATTTACAAG**	**GAAGTTTTCTCCCAATGGGATTTACAAA**	**TGGCTTGTTATCTACTGGTTACACTGTTT**	**G/A**
21552	t7_78	43	AGCTATGCATACAACTCCAATACGC	CAGCTATGCATACAACTCCAATACGT	GGATAGGATGCATGTAAATGAGATCAGTA	G/A
23650	**t7_79**	**34**	**GTCGAGATTCTTGACCACTCACG**	**AGTCGAGATTCTTGACCACTCACA**	**TGAGCTACCCGACTGGGCACTT**	**C/T**
24965	t7_80	40	AAAATCGCGATGATTACAAATGCAGTG	AATAAAATCGCGATGATTACAAATGCAGTT	CCGATCTTAACCCCAAAATGGGTCAT	G/T
30089	**t7_81**	**35**	**AAAGTTGTAAACAGTCAAGTAAAACTTACAAAA**	**AAAGTTGTAAACAGTCAAGTAAAACTTACAAAT**	**GCGTTGGTTTCCTTCGATCTGTCAA**	**T/A**
15395	t7_87	27	CAGGACCAATAAGCTTCAAGTTGGA	AGGACCAATAAGCTTCAAGTTGGG	TCGTTTGACAGCCAAAGGTTTCAGTATT	A/G
20939	**t7_88**	**22**	**GCAGGTGGAATCGTGGCTTCG**	**GCAGGTGGAATCGTGGCTTCA**	**GAGTGTGGACGTGATCATGCAGATT**	**G/A**
22738	**t7_91**	**28**	**GCAGGGAGATTTTGCAAATTAGTATAC**	**GCAGGGAGATTTTGCAAATTAGTATAA**	**GCCCCCAACTGTTCAATGTTAGATTTTTA**	**C/A**
28512	**t7_92**	**40**	**AGTGGTAAAACTAGATAATATACGGGTTA**	**GTGGTAAAACTAGATAATATACGGGTTG**	**CCTAAATGAACCGGTGTTCAA**	**A/G**
15440	**t7_99**	**30**	**ACGTTGACAGGACTAAGCAGC**	**CTACGTTGACAGGACTAAGCAGA**	**CAGGTGGTTTTCCAGGAGGAACAAT**	**G/T**
13319	**t7_58**	**42**	**AACTTCTTAAAAWGATGTTATTATTATGATCG**	**AACTTCTTAAAAWGATGTTATTATTATGATCC**	**GCTGGCTTTTGTTTGTTTGTACCACATTT**	**C/G**
32759	**t7_63**	**38**	**GTCAATTTTGAAGGTACTGAGTTATTTATTG**	**GTCAATTTTGAAGGTACTGAGTTATTTATTT**	**ATAATTACGATTWGTGATAAATTTGCGATA**	**G/T**
10424	**t7_69**	**28**	**CCACTTGGCCTGAGCAATGACTT**	**CACTTGGCCTGAGCAATGACTC**	**TGCAGGAGTACATCCCTGTAAAAGAT**	**A/G**
30252	**t7_93**	**34**	**GGAGATCAGAACTAGAATCCCAGAT**	**GAGATCAGAACTAGAATCCCAGAC**	**CAGGTGCAAAATTRTAGTCCCATGAGAT**	**A/G**
28192	t7_9	37	GTGTTGTATATATATTATTGTTTTTGTCGTTA	GTGTTGTATATATATTATTGTTTTTGTCGTTC	TGCAGGTTGGTTAAATAGTAATATATATAT	T/G
15932	t7_60	24	GCAGGTCATTTTCGGAACCTATA	GCAGGTCATTTTCGGAACCTATT	GAATCCTGTAAGTAACACCGTCTACAA	A/T
1684	t7_64	40	GCAACCATGTCTGGATGCAGC	GCAACCATGTCTGGATGCAGT	GAGTACAACCTGCGCTTGGGTATAA	G/A
9524	t7_68	36	ACCAAGGAGGTGGTGGAGGTTA	ACCAAGGAGGTGGTGGAGGTTT	CCACCGACTTGACCATATTGTCCT	A/T
19440	t7_86	51	GTCCCACAAAACATACAAAAAGGTT	GTCCCACAAAACATACAAAAAGGTA	CAGGTCAGGATTGAATTTCCACCAGTT	A/T
21221	t7_89	49	TGAGTTTTCCCAAACTTGATGTATTT	GAGTTTTCCCAAACTTGATGTATTA	GCAGGATTTTAGGCATCTAAGTCAAACTT	A/T
14567	t7_98	21	GCAGGGGCTTTGGTTCAACC	GCAGGGGCTTTGGTTCAACT	AGCCATTTGAGGGCTGATCCTGTTA	C/T
21241	t7_100	37	CCCATGTTCTTCTTGATGTATTAGTTTC	CCCATGTTCTTCTTGATGTATTAGTTTG	CAGAAGTTCAAAACGCGCTTCATGTTTAT	C/G
9072	t7_55	39	TTTGAATGATAGATTACAAGAGGGGTTTT	GAATGATAGATTACAAGAGGGGTTTC	CTTCATCAGGCTTCATRGCATATCGAA	T/C

Table A.4 Summary of the amplification failure rates for each of the 44 polymorphic SNP loci that were used to genotype 285 *L. fremontii* individuals.

SNP	No. individuals that did not amplify (out of 285)
t7_2	7
t7_5	11
t7_7	3
t7_15	4
t7_16	22
t7_17	4
t7_19	2
t7_21	5
t7_22	13
t7_23	10
t7_26	13
t7_27	8
t7_28	13
t7_29	17
t7_33	16
t7_34	7
t7_36	5
t7_37	18
t7_38	17
t7_39	7
t7_40	16

Table A.4 (Continued)

SNP	No. individuals that did not amplify (out of 285)
t7_42	9
t7_45	3
t7_46	10
t7_49	13
t7_50	9
t7_52	5
t7_56	27
t7_59	34
t7_66	15
t7_67	18
t7_71	40
t7_75	15
t7_77	24
t7_79	8
t7_81	10
t7_88	6
t7_91	24
t7_92	34
t7_99	5
t7_58	4
t7_63	54
t7_69	7
t7_93	14
Average	**14**

Table A.5. Statistical results (P-values) from Hardy-Weinberg equilibrium test per SNP locus and vernal pool complex. Values of P < 0.05 (highlighted) identify loci that were not in HWE. Populations that were monomorphic at a locus (and thus not evaluated for Hardy-Weinberg equilibrium) are indicated by M.

Locus	Vernal Pool Complex									
	DL	NTM	VINA	BAF	MF	GT	JP	BTM	PVP	CARR
t7_2	M	1	1	1	0.23	1	M	0.08	0.54	1
t7_5	M	M	1	M	M	M	M	M	M	M
t7_7	M	M	M	M	M	M	M	1	M	M
t7_15	M	M	M	M	M	M	M	1	M	M
t7_16	0.22	0.04	0.05	0.2	0.46	0	0.31	M	0.17	0.23
t7_17	1	1	1	0.09	M	1	1	M	1	M
t7_19	0.03	M	1	1	M	1	M	M	0.08	1
t7_21	1	1	1	1	0.43	1	1	M	1	1
t7_22	0	0.62	0.18	1	0.32	1	1	0.07	0.23	1
t7_23	0.54	0.18	0.6	1	1	0.37	0.25	0.32	0.57	1
t7_26	M	M	0.1	1	0.6	1	M	M	M	1
t7_27	M	0.18	M	M	1	M	M	0.57	M	M
t7_28	1	1	M	M	1	1	1	M	M	M
t7_29	1	1	1	0.57	1	0.09	1	1	1	1
t7_33	M	M	1	M	M	M	M	M	0.1	M
t7_34	0.46	1	M	M	M	M	M	M	M	M
t7_36	M	0.51	1	M	M	M	M	M	M	M
t7_37	1	1	1	M	1	1	0.16	M	M	M
t7_39	M	M	M	M	M	M	1	M	M	M
t7_40	M	0.3	M	M	M	M	M	M	M	M
t7_42	M	1	M	M	M	1	M	M	M	M
t7_45	1	1	1	0.54	1	1	1	1	1	0.06
t7_46	M	0.01	M	M	M	M	M	M	M	M
t7_49	M	1	M	M	M	M	M	M	M	M
t7_50	1	0.62	0.01	0.62	1	1	0.1	0.11	1	0.34

Table A.5 (Continued)

Locus	Vernal Pool Complex									
	DL	NTM	VINA	BAF	MF	GT	JP	BTM	PVP	CARR
t7_52	1	M	1	M	1	M	1	M	1	1
t7_56	1	0.08	1	1	1	1	0.19	1	0.43	1
t7_59	1	1	0.08	0.1	0.26	0	0.14	0.14	0.07	0.33
t7_66	M	M	M	M	M	M	M	M	M	1
t7_67	M	0	M	0.03	M	M	0.01	M	0	M
t7_71	1	1	M	1	0.22	0.12	M	0.35	M	M
t7_75	1	0.29	1	1	0.22	1	0.48	1	1	M
t7_77	M	1	1	1	0.03	1	1	0.11	0.01	M
t7_79	0	0.32	0	0	0	0	0.02	0	0	0.11
t7_81	M	M	M	M	M	M	M	M	M	1
t7_88	M	1	0.18	1	1	0.17	0.03	M	M	M
t7_91	0.07	0.32	0.59	1	0.51	1	0.18	0.03	1	0.23
t7_92	M	M	1	0.1	M	M	M	0.2	M	M
t7_99	1	0.14	0.09	1	1	1	0.57	0.6	0.65	0.44
t7_58	1	0.04	M	1	M	M	M	M	M	M
t7_63	M	M	M	0.22	M	M	M	M	1	0.23
t7_69	M	M	M	M	M	M	M	1	1	M
t7_93	M	M	1	1	0.11	0.29	0.23	1	1	M
Total # of loci in H-W equilibrium	**18**	**25**	**24**	**24**	**21**	**21**	**21**	**18**	**20**	**19**
Total # of Monomorphic loci	**23**	**13**	**18**	**18**	**21**	**20**	**22**	**24**	**21**	**25**

Table A.6 Statistical results (P-values) of the Hardy-Weinberg equilibrium test performed per locus and pool at Mather Field. Values of P < 0.05 (highlighted) identify loci that were not in HWE. Monomorphic loci (not evaluated for HWE) are indicated with M.

Locus	Pool at Mather Field								
	E12	**E13**	**E25**	**P10**	**P143**	**P160**	**P317**	**P318**	**P74**
t7_2	1	0.16	1	1	0.22	1	1	1	0
t7_5	M	M	M	M	M	M	M	M	M
t7_7	M	M	M	M	M	M	M	M	M
t7_15	1	0.31	M	0.05	0.57	0.05	0.17	0.01	1
t7_16	M	M	1	M	M	0.05	M	M	M
t7_17	M	M	M	M	M	M	M	M	M
t7_19	1	1	M	1	M	M	M	M	M
t7_21	1	M	1	1	1	1	M	0.28	1
t7_22	1	1	1	1	1	0.57	1	0.25	1
t7_23	0.56	1	1	1	1	0.49	1	1	0.01
t7_26	1	1	0.34	1	0.06	1	1	1	1
t7_27	M	1	M	M	M	M	M	1	M
t7_28	1	M	1	M	M	0.05	M	M	M
t7_29	0.22	1	0.25	1	0.38	0.57	1	0.54	1
t7_33	M	M	M	M	M	M	M	M	M
t7_34	M	M	M	M	M	M	M	M	M
t7_36	M	M	M	M	M	M	M	M	1
t7_37	1	1	1	M	1	1	M	1	0.2
t7_38	M	M	M	M	M	M	M	M	M
t7_39	M	M	M	M	M	M	M	M	M
t7_40	M	M	M	M	M	M	M	M	M
t7_42	M	M	M	M	1	M	M	M	M
t7_45	1	1	1	1	1	1	M	M	1
t7_46	M	M	M	M	M	M	M	M	M
t7_49	0.17	1	1	0.53	1	1	1	0.48	0.06

Table A.6 (Continued)

Locus	Pool at Mather Field								
	E12	**E13**	**E25**	**P10**	**P143**	**P160**	**P317**	**P318**	**P74**
t7_52	M	M	M	M	M	M	M	M	M
t7_56	1	0.2	0.48	1	1	1	1	0.54	1
t7_59	M	M	M	M	M	M	M	M	M
t7_66	0.05	0.06	0.48	0.18	M	0.06	0.01	1	M
t7_67	M	M	M	M	M	M	M	M	M
t7_71	M	0.05	M	M	M	0.05	M	M	M
t7_75	0.06	1	M	0.06	0.31	0.02	0.17	1	M
t7_77	1	0.31	1	0.48	0.48	0.24	0.05	1	1
t7_79	1	M	M	M	M	M	0.05	1	1
t7_81	0	0.01	0.17	0	0	0	0.01	0.01	0.06
t7_88	M	M	M	M	M	M	M	M	M
t7_91	1	M	1	M	M	M	1	0.23	1
t7_92	0.48	1	1	0.17	0.56	1	0.48	0.04	1
t7_99	M	M	M	0.05	1	M	0.05	M	M
t7_58	0.48	1	1	0.13	0.5	0.08	1	0.55	0.57
t7_63	M	M	M	M	M	0.06	0.06	M	M
t7_69	M	M	M	M	M	M	M	M	M
t7_93	1	1	1	1	1	1	0.31	1	1
Total # of loci in H-W equilibrium	22	19	20	17	19	21	17	18	17
Total # of monomorphic loci	21	24	24	25	24	22	25	23	25

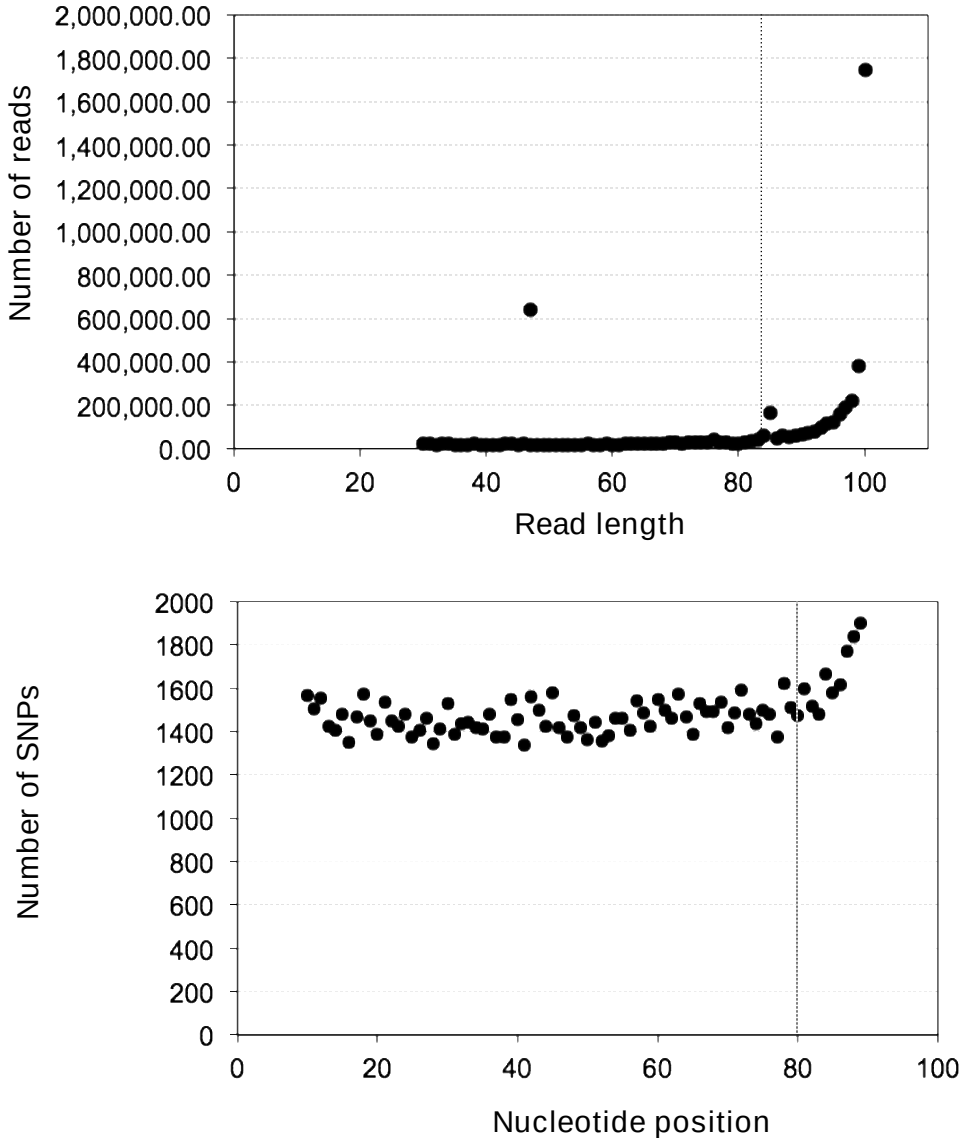

Figure A.1 Top: Ranges of read lengths obtained before quality filtering and their average representation from the total number of reads obtained. The highest number of reads were ≥ 85bp long (identified with the dashed vertical line). Bottom: Preliminary de novo SNP calling (using the default parameters in STACKS v. 1.02_ revealed over-representation of SNPs in the last 20bp region of the reads (light gray dashed line), suggestive of sequencing errors. Based on these results I decided to trim the reads to 75bp because the over-representation of SNPs was at >80bp, therefore I removed an additional 5bp to avoid false SNP discovery at the end of the reads.

Table B.1 Locality information and ownership of the vernal pool complexes where *L. fremontii* samples were collected in 2013. Locations are listed from the most northern to the most southern site. CDFW = California Department of Fish & Wildlife; UCNRS = University of California Natural Reserve System. Latitude and longitude are in units of decimal degrees.

Location Name (abbreviation)	County (CA)	Property Owner	Latitude (N)	Longitude (W)
Dales Lake Ecological Reserve (DL)	Tehama	CDFW	40.32878	-122.06222
Beale Air Force (BAF)	Yuba	United States Air Force	39.60068	-121.54987
Mather Field (MF)	Sacramento	Sacramento County DP&R	38.53748	-121.26267
Jepson Prairie Preserve (JP)	Solano	UCNRS	38.26596	-121.82706
Big Table Mountain Ecological Reserve (BTM)	Fresno	CDFW	37.04526	-119.59592
Pixley Vernal Pool Preserve (PVP)	Tulare	Center for Natural Lands Management	35.98547	-119.21205

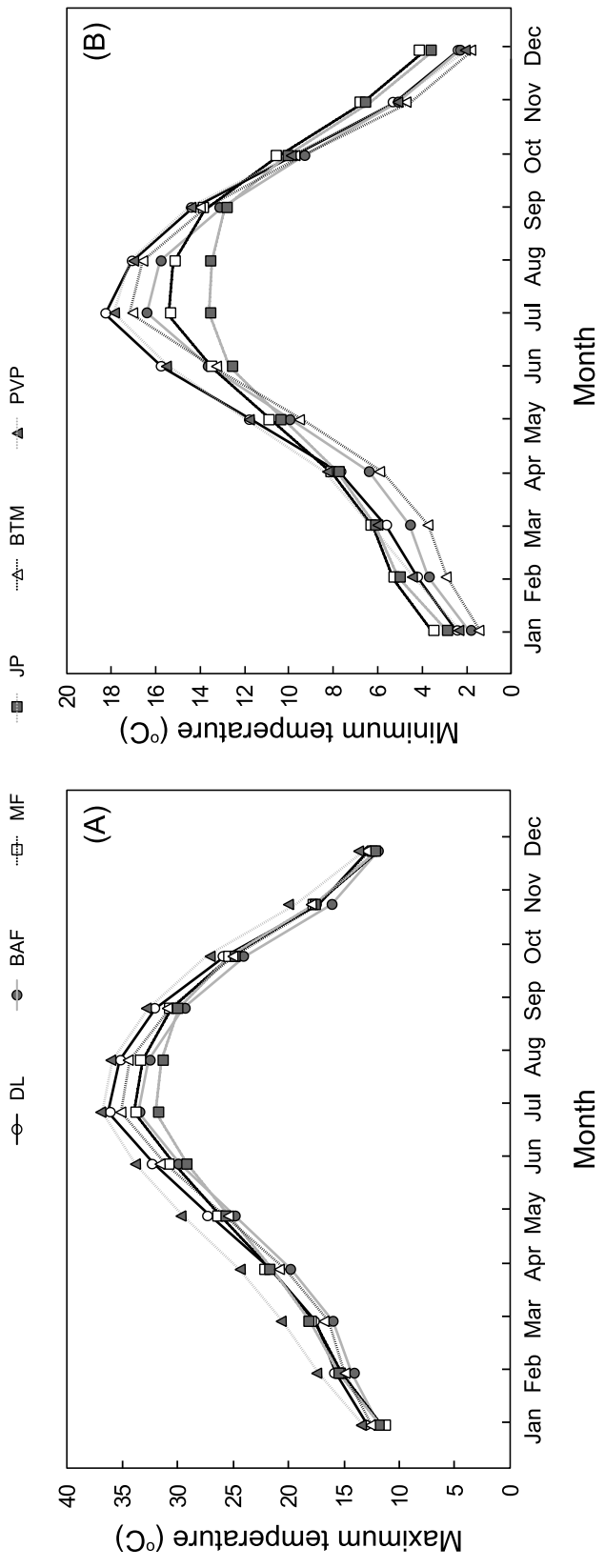

Figure B.1 Average maximum (A) and minimum (B) temperatures for the years 1981-2013 from the geographic locations of the *L. fremontii* populations included in the germination experiment. Data were extracted from the Worldclim database (see Methods). These records showed no strong differences among populations in their local temperature conditions.

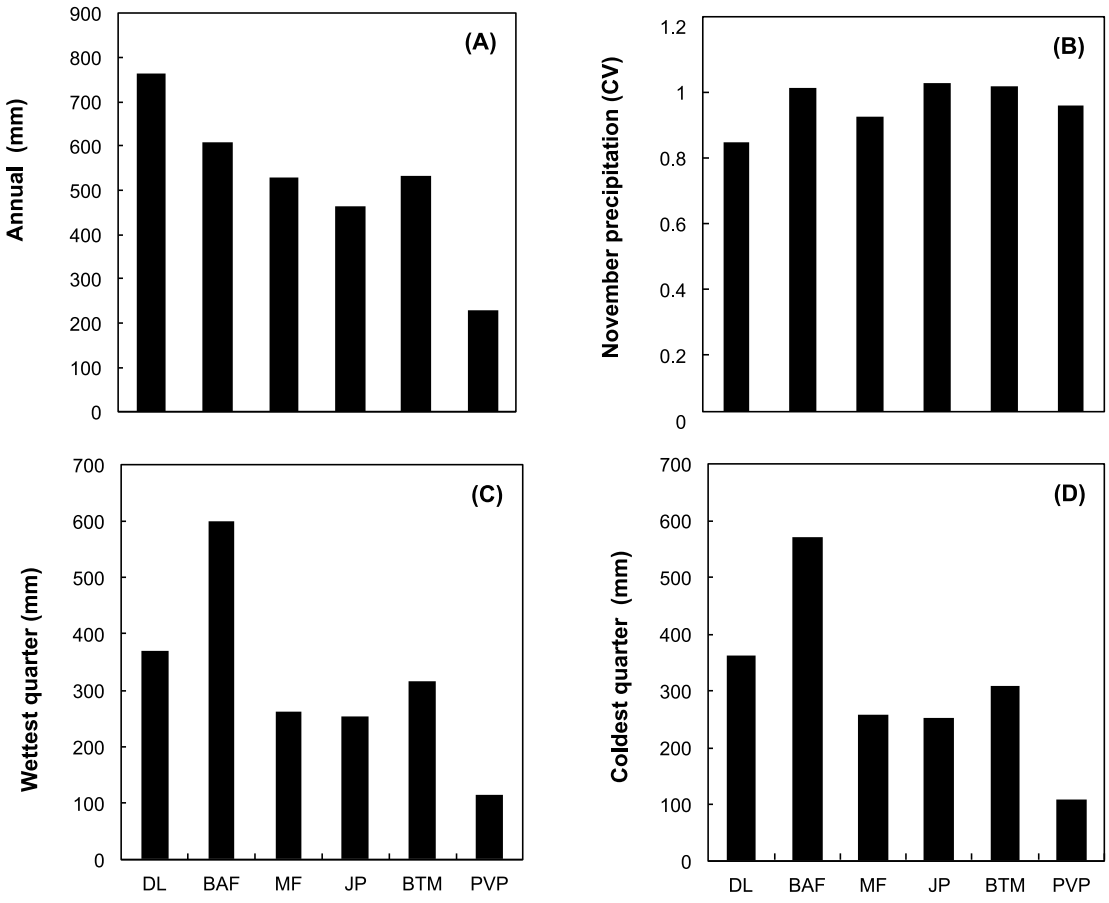

Figure B.2 Precipitation variables used in the principal component analysis to describe the historical precipitation conditions at the geographic locations occupied by the *L. fremontii* populations in this study. Data were extracted from the PRISM database for the years 1981-2013, Worldclim for the years 1950-2000 (see Methods). (A) Average annual precipitation. (B) Historical variation in November precipitation, defined as the variability of rainfall (CV) for vernal pool complexes where *L. fremontii* samples were collected in 2013. (C) BIO16, precipitation during the wettest month. (D) BIO19, precipitation during the coldest quarter.

Appendix C: Additional figures and tables for Chapter 3

Table C.1 Summary of the yearly climatic data that were used to develop the hydroperiod treatments for the greenhouse experiment. I obtained bimonthly MEI indices for winter in California for the past three decades from NOAA (Wolter and Timlim 2011), and identified three years with the highest and lowest MEI values, which indicate the most extreme El Niño and La Niña events, respectively. Three "normal" years were also selected by intermediate values of the MEI index. I then obtained the historical precipitation data for each focal population for all nine years to estimate the hydroperiod using the water balance model (Figure 3.3). The hydroperiod was estimated for each year separately and then I calculated the mean hydroperiod by ENSO event per population.

Year Period	MEI Index (Nov/Dec)	MEI Index (Dec/Jan)	MEI Index (Jan/Feb)	Event
1982-1983	64	65	65	El Niño
1992-1993	48	51	57	El Niño
1997-1998	63	64	64	El Niño
1988-1989	5	7	6	La Niña
2007-2008	9	13	4	La Niña
2011-2012	14	11	17	La Niña
1984-1985	19	22	22	Normal
1993-1994	46	43	39	Normal
2004-2005	47	40	51	Normal

VITA

VITA

Lorena Torres-Martinez, PhD Candidate

CONTACT INFORMATION

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PERSONAL INFORMATION

Date of Birth: April 04, 1986
Place of Birth: Cali, Colombia
Citizenship: Colombia
Visa State: In US, F1
Gender: Female
Languages: Spanish (native speaker), English (fluent)

EDUCATION

2010 – 2016 **PhD Candidate**
Department of Biological Sciences, Purdue University, West Lafayette, IN
- Thesis Research: Evolutionary potential of a dispersal-restricted species in response to climate change
- Faculty Mentor: Dr. Nancy Emery and Dr. Morris Levy

2009 – 2010 **Specialization in Climate Global Change and the Kyoto Protocol**
Latin-American Science Institute, Lima, Peru
- Thesis Research: Estimation of the potential distribution of *Guadua angustifolia* and the effect of climate change under two ecological scenarios for the 2050.

2003 – 2008 **Bachelor of Science, Biology**
Faculty of Natural and Exact Sciences, Department of Biology, Universidad del Valle, Cali, Colombia
- Thesis Research: Evaluation of the Polymorphic Content Information of 26 microsatellites for the Molecular Characterization of the Germ Bank of *Guadua angustifolia*
- Major: Genetics
- Faculty Mentor: Msc. Heiber Carden

FELLOWSHIPS, GRANTS AND AWARDS

2015	Women in Science at Purdue travel grant (WISP) Purdue University
2014	Northern California Botanists Scholarship
2014	National Science Foundation Dissertation Improvement Grant (NSF DDIG)
2013 – 2014	PRF Research Grant. Graduate School, Purdue University
2013	Frederick N. Andrews Environmental Travel Grant. Purdue University
2010	Colombian Association of Biological Sciences Award
2008	Honor Graduate in the Biology program of the Universidad del Valle

PUBLICATIONS

Journal Publications – Nearing Publication

Torres-Martínez, L. and N.C. Emery. Using RADseq to discover SNP markers in the California vernal pool endemic herb, *Lasthenia fremontii* (Asteraceae). *In press. Conservation Genetic Resources*.

Torres-Martínez, L, †P. Weldy, M. Levy and N.C. Emery. Germination variation in Fremont's Goldfields: Implications for vernal pool plant responses to climate change. *Invited for special issue in Annals of Botany.*

Journal Publications- Nearing submission († Undergraduate researchers)

Torres-Martínez, L., McCarten, N., †Hochstedler, B., †Paglia, D., †Williams, S., and N.C. Emery. Plant evolutionary potential for responding to ENSO events: a Californian vernal pool endemic perspective. *In preparation* for submission to *Global change biology.*

Torres-Martínez, L., McCarten, N. and N.C. Emery. Historical local climate influences the expression of phenotypic plasticity in response to extreme climate events. *In preparation* for submission to *Evolution.*

Torres-Martínez, L., Navarro, L and N.C. Emery. Gene flow by seed and pollen dispersal: implications for plant adaptation to a changing climate. *In preparation* for submission to *Molecular ecology*.

Emery, N.C., †M. Madden, and **L. Torres-Martínez**. The evolution of reaction norms and habitat affinity: hydrological tolerance curves and wetland specialization in Lasthenia. *In preparation* for submission to *Functional Ecology*.

Book Chapters

Schuster M., **Torres Martínez L.**, Dukes J.S. 2012. Distribution of terrestrial ecosystems and changes in plant community composition. In: Freedman B. (Ed.) Global Environmental Change. Handbook of Global Environmental Pollution. Springer Netherlands, pp. 341-347. DOI: 978-94-007-5783-7

Emery, N, **L. Torres-Martínez,** E. Forrestel, B.G. Baldwin and D.D. Ackerly. 2011. The ecology, evolution and diversification of the Vernal Pool Niche in Lasthenia (Madieae, Asteraceae) p 39- 57. In: Alexander, D.G and R.A. Schlising. 2011. Research & Recovery in Vernal Pool Landscapes. Studies from the Herbarium. Number 16. California State University, Chico.

Bocanegra, JL, C. Villafañe, R. Moreno, **L. Torres-Martínez**, A. Velásquez, L. Fory and G. Gallego. 2010. Genetic Characterization of wild cultivars and species of yucca (Manihot sp.) on the Colombian Amazon and Orinoco. In: Capacity building for implementation of the Protocol of Cartagena in Colombia: Sector Ambiente - / Instituto de Investigación de Recursos Biológicos Alexander von Humboldt; Orjuela-R. M.A. y Moreno V. R. (comp.). Bogotá D.C.: Colombia: Ministerio de Ambiente, Vivienda y Desarrollo Territorial; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. 2010.

PRESENTATIONS

Invited presentations

Emery, N.C. and **L. Torres-Martínez**. 2015. Evolution of phenotypic plasticity and ecological specialization in temporally varying environments. Organized oral session: "Shifting dimensions: temporal ecology for the next 100 years and beyond." Ecological Society of America Annual Meeting, Baltimore, MD.

Emery, N.C. and **L. Torres-Martínez**. 2015. Rapid Evolution and Phenotypic Plasticity of Vernal Pool Plants in Response to Climate Change. Symposium title: "Best Management Practices for Climate Change Adaptation: A Wetlands Perspective." Society for Wetland Scientists, Providence, RI.

Torres-Martínez, L. and N.C. Emery. 2014. Germination and Dormancy Variation in Fremont's Goldfields: Implications for Vernal Pool Plant Responses to Climate Change. Northern California Botanists Symposium. California State University, Chico, CA.

Torres-Martínez, L., D. Lopez, C. Perez-Galindo, M.C. Duque, I.A.Gonzalez, J.D. Palacio and H. Cardenas.2009. Molecular Characterization with microsatellites markers of the Juan Maria Cespedes Botanical Garden germ bank accessions of *Guadua angustifolia* (Poaceae:Bambusoideae). International Congress of Guadua, other Bamboos and Natural Fibers. Armenia, Colombia.

Contributed presentations († Undergraduate researchers)

Torres-Martínez, L and N.C. Emery. 2015. The spatial scale of genetic differentiation in wetland plant populations: implications for adaptation to changing climate. Evolution. Guaruja, Sao Pablo, Brazil.

Emery, N.C., **L. Torres-Martínez,** and †M. Madden. 2013. Reaction norm evolution and habitat specialization in California goldfields (Lasthenia, Asteraceae). Oral Presentation, Evolution. Snowbird, UT.

Torres-Martínez, L., D. Lopez, C. Perez-Galindo, M.C. Duque, I.A.Gonzalez, J.D. Palacio and H. Cardenas. 2009. Evaluation of the polymorphism of microsatellites markers in *Guadua angustifolia* (Poaceae: Bambusoideae). Poster presentation. VIII World Bamboo Congress. Thailand, Bangkok.

Torres-Martínez, L., D. Lopez, C. Perez-Galindo, M.C. Duque, I.A.Gonzalez, J.D. Palacio and H. Cardenas.2009. Molecular Characterization with microsatellites markers of the Juan Maria Cespedes Botanical Garden germ bank accessions of *Guadua angustifolia* (Poaceae:Bambusoideae). Poster presentation. V Colombian Congress of Botany. Nariño University, Pasto, Colombia.

WORKSHOPS

2015	Workshop in conservation genomics. Lakretz Field Station. University of California, Los Angeles, CA, USA. March 22-27, 2015
2011	Practical computing for biologist. NESCENT. North Carolina State University, Raleigh, NC, USA. June 6-1, 2011
2010	VI Workshop of Conservation genetics: micro-evolutionary processes in wild species. Latin-American network of conservation genetics (REGENEC). Chillán, Chile. January 12-22, 2010

2009	Course in Phylogeography: sequence and microsatellites data analyses. Universidad del Valle, Biological Sciences Department, Cali, Colombia. June 22-27, 2009
2007	Workshop in phylogenetic applications of flow cytometry. II Latin-American Symposium of Cytogenetics and Evolution. Universidad Nacional de Colombia. Palmira, Colombia. August 15-18, 2007

TEACHING EXPERIENCE

2016	**Teaching Assistant, BIOL 580 (Evolution).** Department of Biological Sciences, Cluster of Ecology and Evolution. Purdue University, West Lafayette, IN. Instructor: Dr. Morris Levy.
2015	**Teaching Assistant, BIOL 110 (Biology).** Department of Biological Sciences, Cluster of Ecology and Evolution. Purdue University, West Lafayette, IN. Instructor: Dr. Athena Anderson.
2013	**Teaching Assistant, BIOL 483 (Conservation Biology).** Department of Biological Sciences, Cluster of Ecology and Evolution. Purdue University, West Lafayette, IN. Instructor: Dr. Kerry Rabenold.
2011 – 2012	**Teaching Assistant, BIOL 580 (Evolution).** Department of Biological Sciences, Cluster of Ecology and Evolution. Purdue University, West Lafayette, IN. Instructor: Dr. Morris Levy.

UNDERGRADUATE MENTORING (RESEARCH TRAINEES)

Alan Clinton	Honor Thesis Mentoring. Development and characterization of microsatellites in *Lasthenia*.
Shannon L. Kuznar	Honor Thesis Mentoring. Dispersal trait variation in *Lasthenia fremontii.*
Nicholas G. Barton	Department of Biological Sciences, Senior student, Major: Ecology, Evolution, and Environmental Biology. Trained in DNA extractions; ecological data collection and analysis; experimental design in germination and quantitative genetics experiments.
Wanyu Huang	Department of Chemistry. Senior student. Major: Biochemistry. Trained in: DNA extractions; ecological data collection and analysis; experimental design of quantitative genetics experiments.
Suraya Williams	Department of Biological Sciences, Junior student, Major: Ecology, Evolution, and Environmental Biology. Trained in: DNA extractions, ecological data collection and analysis; experimental design in germination and quantitative genetics experiments.

Phillip Weldy	Department of Biological Sciences, Junior student, Major: Ecology, Evolution, and Environmental Biology. Trained in: DNA extractions; ecological data collection and analysis; experimental design in germination and quantitative genetics experiments.
Baylie Hochstedler	Department of Biological Sciences, Junior student, Major: Biology Health and Disease. Trained in: DNA extractions; ecological data collection and analysis; experimental design in germination and quantitative genetics experiments.
Ching-Hui Wu	Department of Botany and plant pathology. Major: Botany. Trained in ecological data collection.
Brittany R. Croy	Department of Biological Sciences, Junior student, Major: Ecology, Evolution, and Environmental Biology. Trained in: DNA extractions and PCR amplification.
Megan Sullivan	Department of Biological Sciences, Junior student, Major: Biology Health and Disease. Trained in: DNA extractions and PCR amplification; ecological data collection and analysis.
Danielle Paglia	Department of Biological Sciences, Junior student, Major: Biology Health and Disease. Trained in: ecological data collection and analysis; experimental design in germination and quantitative genetics experiments.

WORK EXPERIENCE

2009 – 2010 **Researcher and Tissue collection curator** at the Tissue Collection of Alexander Von Humboldt Biological Resources Research Institute (CIAT-CGIAR, Cali-Colombia).

- Molecular Characterization of Cassava, Rice and its wild relatives in Colombia (Biosafety- Biological Collections of Wild relatives of Modified Organism).
- Processing and tagging of plant and animal tissue for cryogenic conservation of Colombian Biodiversity

2009 **Researcher,** Estimation of the Genetic Diversity of the Populations of *Guadua angustifolia* (American Bamboo) in the Valle del Cauca-Colombia. Universidad Santiago de Cali/Alexander Von Humboldt Biological Resources Research Institute.

2009 **Researcher,** Genetic resemblance between *Guadua* sp. Universidad Santiago de Cali/ Institute for Research and Preservation of Cultural and Natural Heritage of Valle del Cauca, INCIVA / Alexander Von Humboldt Biological Resources.

2007 – 2008	**Researcher,** Evaluation of the Polymorphic Content Information of 26 microsatellites for the Molecular Characterization of the Germ Bank of *Guadua angustifolia*. Universidad del Valle/Universidad Santiago de Cali/ Institute for Research and Preservation of Cultural and Natural Heritage of Valle del Cauca, INCIVA/ Alexander Von Humboldt Biological Resources Research Institute.
2007	**Laboratory Technician,** Development of the first DNA bank of *Guadua angustifolia* (American Bamboo). Universidad Santiago de Cali / Institute for Research and Preservation of Cultural and Natural Heritage of Valle del Cauca, INCIVA.

SKILLS

WET LAB

- Experience in plant and animal tissue DNA extraction, PCR amplification, RADseq library construction.
- Expertise in Chemical safety and reagent preparation in a molecular biology laboratory.
- Experience with plant and animal tissue collection and long-term storage under cryogenic conditions while managing the unique life tissue collection of Colombian Biodiversi

COMPUTATIONAL AND STATISTICAL EXPERTISE

- Ability to develop pipelines in perl and R to analyze next-generation sequencing data Experience with STACKS (RADseq analysis), bowtie, bwa, samtools, GATK, Abyss , Velvet and other software for genome assembly and SNP calling.
- Experience in statistical analysis of big datasets using SAS and R languages.
- Experience in niche modeling using Maxent (bioclimatic envelope models)

FIELD SAMPLING AND GREENHOUSE EXPERIENCE

- Development, coordination and management of big planting experiments in both growth chambers and green houses.
- Experience in sampling and collection of plant tissue for DNA extraction in both tropical (cloud forest) and temperate ecosystems (vernal pool wetlands).
- Field base collection of birds and bats diversity inventory in tropical natural preserves.

MEMBERSHIP IN PROFESSIONAL SOCIETIES

2015 – present	Society for the study of evolution
2014 – present	Northern California Botanist
2012 – 2013	Secretary, Colombian Student Association at Purdue (CSAP)
2011 – present	SACNAS (Purdue Chapter)