

PLANT EVOLUTIONARY RESPONSE TO CLIMATE CHANGE: DETECTING
ADAPTATION ACROSS EXPERIMENTAL AND NATURAL
PRECIPITATION GRADIENTS

by

Jacqueline J. Peña

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

Approved:

Peter B. Adler, Ph.D.
Major Professor

Zachariah Gompert, Ph.D.
Committee Member

Kari E. Veblen, Ph.D.
Committee Member

Laurens H. Smith, Ph.D.
Interim Vice President for Research and
Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2018

Copyright © Jacqueline J. Peña 2018

All Rights Reserve

ABSTRACT

Plant Evolutionary Response to Climate Change: Detecting Adaptation Across
Experimental and Natural Precipitation Gradients

by

Jacqueline J. Peña, Master of Science

Utah State University, 2018

Major Professor: Dr. Peter B. Adler
Department: Wildland Resources

Forecasting how populations respond to global change is an ongoing challenge. Current approaches using population dynamic models ignore the potential for evolutionary adaptation. Evidence for eco-evolutionary dynamics suggests that such models might under or overestimate the impacts of climate change. Using a population genomics approach, we genotyped individual plants in a 5-year precipitation manipulation experiment in the sagebrush steppe to investigate short-term genetic changes. Focusing on the perennial bunchgrass, *Pseudoroegneria spicata*, we asked, is there genetic variation under experimental precipitation gradients, and are changes in genetic variation in response to experimental precipitation manipulations consistent with spatial patterns in genetic variation along natural elevation gradients? The precipitation experiment consists of three treatments of 1m² quadrats consisting of: control, drought with 50% ambient precipitation, and irrigation with 150% ambient precipitation. In addition, we established one low and one high elevation site to compare overall levels of

genetic diversity between experimental and natural precipitation regimes. Leaf tissues were collected from all precipitation treatments to isolate plant DNA. We used genotype-by-sequencing protocols to obtain DNA libraries and sequence data was used to identify variable nucleotides from de novo assembly. Genotype and allele frequencies were estimated using a hierarchical Bayesian model. We calculated the expected heterozygosity, F_{st} , and quantified the number of SNPs that were associated between the experimental and elevation treatments. We found that genetic variation was similar between drought and irrigation. In contrast, along the natural precipitation regimes, there was slightly more genetic variation in the high elevation site, but this was still low. F_{st} was low overall at the experimental treatment level, but when examining genetic differentiation across all loci there was stronger differentiation between low and high elevation sites. We found an excess number of SNPs associated with the experimental and elevation treatments than would be expected by chance. Overall, our results show some evidence of selection at the loci level and this was consistent between our experimental and elevation treatments. Our study indicates the potential for adaptive responses to drought over short time scales.

(40 pages)

PUBLIC ABSTRACT

Plant Evolutionary Response to Climate Change: Detecting Adaptation Across
Experimental and Natural Precipitation Gradients

Jacqueline J. Peña

Global climate change is a real-time problem that presents threats to many species. Climate change can alter ecosystems and may lead to species extinction. Species can respond to climate change by moving to a better environment or adapting. Therefore, it is necessary to rely on several approaches and perspectives to anticipate ecological impacts of climate change. A common strategy uses models to understand how populations respond to different climate scenarios. Ecological models have helped us understand population persistence, but they often ignore how populations adapt to environmental stress. Adaptive evolution has been ignored because it was assumed that evolution was too slow to have any effect on ecology. Current research has shown that some populations are able to rapidly adapt to novel environments and this is essential for population persistence. We used a population genomics approach to understand how different precipitation regimes affect the perennial bunchgrass, *Pseudoroegneria spicata*, in the eastern Idaho sagebrush steppe. Our objective was to determine how genetic diversity changes under manipulated precipitation regimes and whether these changes were consistent with patterns of genetic diversity under natural precipitation regimes. The manipulated precipitation regimes consist of three precipitation treatments: control, drought with 50% ambient precipitation, and irrigation with 150% ambient precipitation. The natural precipitation regimes consist of two treatments: low elevation (drier than the

experimental site) and high elevation (wetter). We collected plant tissue to isolate plant DNA and then used sequenced DNA for analyses. We used a hierarchical Bayesian model to estimate genotypes and allele frequencies across all loci. We found that there were low levels of genetic variation across all experimental precipitation treatments. When examining genetic differentiation, we found there was stronger differentiation in the natural precipitation regimes. Our study focuses on the short-term responses to climate to understand how environmental stress influences genetic diversity.

ACKNOWLEDGMENTS

I would like to thank my major professor Dr. Peter B. Adler for his patience and support. Peter was a great mentor because he always gave me insightful advice not only about life but also about how to be a better scientist. He challenged me intellectually and was there for me when I needed help or a shoulder to cry on. Peter always encouraged me, and I felt that he never lost faith in me even when I doubted my own capabilities.

I would like to thank Dr. Zachariah Gompert for sharing his vast brilliant knowledge about evolutionary biology and for his patience. Zach helped me to transition into a new research field, population genomics. He always gave me insightful articles and books to read that strengthen my understanding of evolution. I appreciated that Zach always gave me advice about the research life. My favorite thing about Zach was his laughter and his positivity.

I would also like to thank my committee member Dr. Kari Veblen for her service on my committee. I thank past and current members of the Adler lab: Andy Kleinhesselink, Dani Smull, Andrew Tredennick, Britta Teller, and Anny Chung. I would like to give a special thanks to Andrew Tredennick for being my R hero. I appreciated how he explained the R code to me and he always encouraged me. I am grateful for the emotional support Dani Smull gave me when I was new to the Adler lab. Dani was more than a colleague, she was a friend that helped me transition living in a new place. I thank members of the Gompert lab: Lauren Lucas, Amy Springer, Samridhi Chaturvedi, Tara Saley, and Alex Rego. Thank you, Amy and Sam, for explaining concepts in evolutionary biology more clearly to me and helping me out whenever I needed it. Thank you, Amy,

Sam, and Tara, for being supportive friends. I thank Dr. Noelle Beckman for your emotional support and for giving me great advice. Thank you, USU school of graduate studies for your graduate training series, I learned a lot from your workshops that helped to me navigate graduate school better. I thank Dr. Anita Orendt and the Center for High Performance Computing at the University of Utah for providing the computational resources for my thesis.

This project was funded by two main sources of funding. I received funding from a NSF grant that was awarded to Dr. Peter B. Adler that supported me as a research assistant and for funding from the Department of Biology that supported me as teaching assistant. I am appreciative of all these funds because without them my thesis would not be completed.

Lastly, I give special thanks to my family for their support. I am grateful for my dad who always pushed me to be resilient and for his tough love. I thank my mom for being my rock throughout this process. My mom always knew how to encourage me. I thank my sister for being the sunshine in my life, she knew how to make me smile even on bad days. I thank my boyfriend, for being my best friend and for always supporting me in all aspects of my life. I appreciate that he likes it when I geek out about my research and for pushing me to pursue my research dreams.

Jacqueline J. Peña

CONTENTS

	Page
ABSTRACT	iii
PUBLIC ABSTRACT	v
ACKNOWLEDGMENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
INTRODUCTION	1
METHODS	4
RESULTS	13
DISCUSSION	20
CONCLUSION	23
LITERATURE CITED	24

LIST OF TABLES

Table		Page
1	Information about the number of <i>P. spicata</i> plant individuals collected from all precipitation treatments at USSES June 2016	12
2	Number of genets and genet turnover from the start of the precipitation experiment in 2011 through 2016.....	15
3	Table showing the 95-98.5 th percentile range for the observed shared overlap of the SNPs between the experimental and elevation precipitation treatments	19

LIST OF FIGURES

Figure	Page
1	Minor allele frequency across all precipitation treatments15
2	The average expected heterozygosity with 95% confidence intervals of all precipitation gradients.....16
3	F_{st} distributions across all loci among the experimental (top) and elevation precipitation treatments (bottom).....17
4	F_{st} of the experimental (drought and irrigation, D*I) treatments against F_{st} of the elevation (high and low elevation, H*L) treatments18
5	Line plot showing the x-fold enrichments across the 95-98.5 th percentile range for the shared overlap of SNPs between the experimental and elevation precipitation treatments.....19

INTRODUCTION¹

Understanding how populations respond to global climate change is an ongoing challenge. Populations can respond by migrating to better habitats or adapting to altered environments (Lafontaine et al. 2018). A key question is why certain populations are more sensitive, while other populations persist. Through ecological forecasting, we can predict how populations will respond to climate change. By modeling species distribution and abundance, we can characterize population dynamics through space and time (Ehrlén and Morris 2015). For instance, we can do so by linking demography with weather to quantify how climate influences the state of populations (Dalglish et al. 2011, Adler et al. 2012, 2013, Kleinhesselink and Adler 2018). However, these models typically ignore the potential for adaptive evolution (Hoffmann and Sgrò 2011, Coulson et al. 2017), meaning that they may over or underestimate how sensitive populations will be to climate change.

Ecological forecasts ignore adaptive evolution because it has been assumed that evolution is too slow to influence ecological dynamics. However, empirical studies in eco-evolutionary dynamics have challenged this assumption, and it is well established that evolution and ecology can occur on the same time scale (Carroll et al. 2007, Fussmann et al. 2007, Pelletier et al. 2009, Hendry 2016). In a changing climate, understanding adaptive evolution via natural selection will inform how populations persist (Bell and Gonzalez 2011, Lindsey et al. 2013, Gomulkiewicz et al. 2017) and may be necessary to rescue a declining population from going extinct (Gomulkiewicz and Holt

¹ Coauthor with Peter B. Adler and Zachariah Gompert

1995, Bell and Gonzalez 2009, Orr and Unckless 2014, Bell 2017). With increasing advances in next generation sequencing, we can examine how genetic diversity changes due to environmental stress, helping identify the genomic basis of adaptation (Anderson et al. 2011, Savolainen et al. 2013, Josephs et al. 2017, Wadgyman et al. 2017). A population genomics approach will inform how climate may influence changes to genetic diversity within populations (Barrett et al. 2008, Orr 2009, Parchman et al. 2012, Savolainen et al. 2013).

Adaptive evolution and genetic diversity within a population are critical for the longevity of a population (Lande and Shannon 1996) and we can use genetic diversity to understand how populations respond to environmental stress. Variation in a population provides the necessary raw material for natural selection through spontaneous new mutations or through alleles already present in the population, i.e., standing genetic variation. Populations need to keep up with climate change, and standing genetic variation is faster and more likely to prevent extinction (Hermisson and Pennings 2005, Barrett and Schluter 2008, Orr and Unckless 2008, 2014). Standing genetic variation may be a mechanism for rapid adaptation to novel environments (Gompert et al. 2012, 2014b, Jordan et al. 2017) and genetic diversity may decrease due to environmental stress (Lande and Shannon 1996, Lindsey et al. 2013, Franks et al. 2016).

Here we use a population genomic approach in an on-going precipitation experiment to determine how climate change may affect overall levels of genetic variation. We worked in a sagebrush plant community in eastern Idaho to observe how climate affects plant populations. We have been recording long-term observations for the past ten years to track plant demography (survival, growth, and recruitment) and model

population and community dynamics (Adler et al. 2009, Dalgleish et al. 2011, Adler et al. 2012, Chu et al. 2016, Kleinhesselink 2017, Tredennick et al. 2018). However, our models do not consider the potential for adaptive evolution and may over or underestimate future climate impacts on populations. By taking advantage of an existing precipitation manipulation experiment, we studied how water availability influences genetic variation. If there are short-term changes in genetic variation, then our current ecological models may not accurately predict how plant populations will respond to climate change.

Our objective was to describe genetic variation under experimental and natural precipitation regimes. We examined how a perennial bunchgrass, *Pseudoroegneria spicata*, responds to drought, irrigation, and natural precipitation gradients to ask: (1) Are there changes in genetic diversity under drought and irrigation treatments? (2) Are experimental changes in genetic diversity and in the frequency of specific alleles consistent with spatial patterns along natural precipitation gradients? We hypothesized that: (1) drought may decrease overall genetic diversity by causing loss of drought insensitive genotypes, (2) there will not be any differences between irrigation and ambient precipitation in genetic diversity unless recruitment of drought insensitive genotypes occurs, and (3) patterns of genetic diversity and the frequency of specific alleles found in drought and irrigation regimes will reflect patterns of genetic diversity along natural precipitation gradients.

METHODS

Study area

Our precipitation manipulation experiment was conducted at the USDA-ARS Sheep Experiment Station (USSES) north of Dubois, Idaho, USA (44.2° N, 112.1°W, 1690 m above sea level) in a sagebrush steppe community. The vegetation is dominated by three-tip sagebrush *Artemisia tripartita*, the perennial forb *Balsamorhiza sagittata*, and three C₃ perennial bunchgrasses *Pseudoroegneria spicata*, *Poa secunda*, and *Hesperostipa comata*. This plant community grows on coarse-textured sandy to stony soils in a semi-arid environment that is characterized by warm summers and cold winters (Tirmenstein 1999). Ecologists at USSES monitored 26 1-m² annually between 1926 and 1957. Vegetation was mapped within each quadrat using a pantograph (Blaisdell 1958) to record basal cover of grasses and canopy cover of shrubs. In 2007, members of the Adler lab relocated 14 of the original quadrats found within permanent livestock enclosures and began remapping the quadrats using the established pantograph method. Six of these historical permanent quadrats in the largest enclosure were used as control quadrats (ambient precipitation) for the current precipitation experiment.

In 2011, members of the Adler lab established 16 additional 1-m² quadrats located in the same enclosure as the 6 control quadrats. The new quadrats were established in pairs and randomly assigned to precipitation addition (irrigation) or precipitation reduction (drought). We avoided establishing the new plots on steep slopes, areas with greater than 20% bare rock cover, and areas with greater than 10% *Purshia tridentata* and *Amelanchier utahensis* cover. Our precipitation experiment consisted of 6 control

quadrats, 8 irrigation quadrats, and 8 drought quadrats, for a total of 22 quadrats. During the duration of our precipitation experiment (2011-2016), the mean annual precipitation was 250 mm year⁻¹ and the mean monthly temperature in January to July ranged from -5.2 °C to 21.8 °C (Kleinhesselink 2017, Tredennick et al. 2018).

We used rain-out shelters and automatic irrigation systems to manipulate ambient precipitation. 2.5 x 2 m rain-out shelters using transparent acrylic shingles intercepted approximately 50% of incoming ambient precipitation, which was channeled into 75 L containers. Collected precipitation was pumped out of the containers and sprayed onto the nearby irrigation plot by two suspended sprinklers. Automatic pumping was triggered by float switches when water levels reached about 20 L. Each year, irrigation pumps were disconnected in October and reconnected in April while rain-out shelters remained in place throughout the year.

Natural precipitation gradient

To complement the precipitation experiment with a natural gradient of ambient precipitation, we established one site at lower elevation (44.20° N, 112.18°W, 1624 m above sea level) and one site at a higher elevation (44.28° N, 112.12°W, 1756 m above sea level). The mean annual precipitation for the low elevation site was 325 mm year⁻¹ and the mean monthly temperature in January to July ranged from -2.06 °C to 12.87 °C (Kleinhesselink 2017). The mean annual precipitation for the high elevation site was 417 mm year⁻¹ and the mean monthly temperature in January to July ranged from -2.64 °C to 12.0 °C (Kleinhesselink 2017). The low elevation site has similar vegetation and soil characteristics as the experimental precipitation site, while the high elevation site is

dominated by mountain big sagebrush, *A. vaseyana*, which occurs at higher elevations and receives more precipitation than three-tip sagebrush.

Our goal was to compare the observed changes in allele frequencies in the experimental treatments to changes in allele frequencies between plant populations at low (drier) and high (wetter) sites. If these changes in allele frequencies are consistent between the experimental and elevation treatments, then this would increase our confidence that *P. spicata* plant populations in our experimental plots are responding to altered precipitation, and not just displaying genetic drift.

Data collection

We collected *P. spicata* leaf-blade tissue of all individuals located in the experimental treatment quadrats, and from individuals at the low elevation and high elevation sites (Table 1). The plant individuals collected from the experimental treatment quadrats were marked on quadrat maps next to the location of the corresponding polygon. The plant individuals collected from the low and high elevation sites were collected on four 100 m transects running parallel to the slope. All collected plant tissue was preserved in silica gel (distributed by Fisher Scientific) for storage.

DNA extraction and genotype-by-sequencing (GBS)

Approximately 50 mg of dried plant tissue was ground in a TissueLyser with tungsten carbide beads. We isolated and purified plant DNA using DNeasy 96 Plant Kit (Cat. No. 69181, Qiagen Inc., Valencia, CA, USA) followed by the manufacturer's protocol for *P. spicata* individuals for each treatment. The final DNA extracted product was stored at -20°C. Genomic libraries were obtained using a genotype-by-sequencing

(GBS) approach to attain partial genome sequences (Parchman et al. 2012, Gompert et al. 2012, 2014a). Each plant's genome was digested with restriction enzymes, EcoRI and MseI, and incubated for 2 hours at 37°C, followed by the inactivation of the enzymes at 65°C for 20 minutes in a thermalcycler. Then double stranded adaptor oligonucleotides were ligated to the digested fragments. The adaptors contain Illumina adaptors necessary for sequencing and unique 8-10 base pair (bp) identification barcode sequence. The fragments were amplified using two separate polymerase chain reactions (PCR) and purified. The PCR primers used were Illpcr1 (forward strand: 5' A*ATGATACGGCGACCACCGAGATCTACTCTTTCCCTACACGACGCTCTTC CGATC*T 3') and Illpcr 2 (reverse strand: 5' C*AAGCAGAAGACGGCATAACGAGCTCTTCCGATCTGTA*A 3'). The first PCR amplification used the primers to amplify DNA fragments that contained the barcodes and adaptors at 98°C for 30 seconds, 30 cycles of 98°C for 20 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and the final extension at 72°C for 10 minutes in a thermalcycle. The second PCR step added the primers, deoxynucleotide solution (dNTPs), and iproof buffer to convert the single stranded DNA template from the first PCR step to double stranded DNA at 98°C for 3 minutes, 60°C for 2 minutes, and 72°C for 10 minutes in a thermalcycle. All individuals were pooled together per each 96-well plate (5 total 96-well plates) for fragment size analysis and fragment sizes of 280-480 bp were excised using Sage Science Blue Pippin at Utah State University Center for Integrated Biosystems (Logan, UT, USA). The final DNA libraries were sequenced at the University of Texas Genomic Sequencing and Analysis Facility (Austin, TX, USA) on the Illumina HiSeq 4000 platform. One Illumina lane of 150 bp single-end reads were

generated.

De novo assembly and alignment

GBS data was assembled *de novo* to identify variable nucleotides for downstream population genomic analyses. We used the computer cluster at The Center for High-Performance Computing (CHPC) at the University of Utah for all computational resources. We split the original fastq files by individual and parsed them. Then we extracted unique sequences from each fastq file and converted them to fasta files. We then concatenated the fasta files to extract the unique reads across all individuals.

We used the clustering program *cd-hit-est* from the *cd-hit* software version 4.7 (Fu et al. 2012) and custom Perl scripts version 5.18.1 to generate a reference genome. We only worked with sequences that were present in at least two individuals and used a hierarchical clustering approach. The first iteration of clustering was used at three different minimum match percentages at 90, 92, and 94 of all sequences against each other. Then a second iteration of the clustered sequences (based on the three different minimum match criteria listed above) were clustered against each other at two different minimum match percentages at 80 and 90 to cluster highly similar clusters based on the centroid of the cluster. The final set of sequences used was the clustered sequences at a minimum match of 90% with a match of clustering of clusters at 80%. We removed clusters that had a low mean sequence similarity, high variation in sequence similarity, and that had a cluster of one. Based on this criterion, we found 509,775 clusters that constructed the reference sequence for downstream alignment.

The final set of consensus DNA sequences were then used for alignment that

contained at least two reads. We used custom Perl scripts and *bwa-mem* command from *bwa* version 0.75a-r405 (Li and Durbin 2009) based on the two rounds of clustering to align the set of consensus sequences to the reference. The *bwa* software was used as a reference-based alignment algorithm to map low-divergent sequences against the reference sequence generated. All bases were trimmed with a phred-scaled quality of less than 30 with a seed length of 15. We then used *samtools* and *bcftools* version 0.1.19 (Li et al. 2009) to compress, sort, and index the sam alignment files and this generated sorted bam files.

Variant calling

SNPs were called using *samtools* and *bcftools* version 0.1.19 (Li et al. 2009) with a full prior set to 0.001. We only called variants when at least 50% of individuals had sequence data that covered a nucleotide position and when the posterior probability of invariant nucleotides was less than 0.01. We then filtered out low quality SNPs using *bcftools* and custom Perl scripts for all individuals to remove only those with a maximum of 20% no sequence data with a minimum coverage of 1,234 sequences on a phred-scaled mapping quality of 30 and with a maximum proportion of reads in reverse order of 0.01. After filtering, we identified 21,581 bi-allelic SNPs that were used for subsequent analyses. The average sequencing depth for the SNPs was 3.36 (SD = 2.28) per individual.

Genotype and allele frequencies

We used a hierarchical Bayesian model described by Gompert et al. (2015) to estimate genotypes (g) and population allele frequencies (p). For each plant individual,

the probability of the sequenced data (x) was conditional on each genotype and the nucleotide quality scores (ϵ), (see Li 2011 for full approach). The prior probability of the plant individual genotype was a function of the population allele frequencies under Hardy-Weinberg expectations: $\Pr(g_{ij}/p_j) \sim \text{binomial}(p_i, n = 2)$. Here, g_{ij} is the individual genotype i at SNP j and denotes the number of nonreference alleles. g_{ij} is 0 if individual i is homozygous for the reference allele, 1 if individual i is a heterozygote, and 2 if individual i is homozygous for the nonreference allele. Here, p_i is the frequency of the reference allele in the population. We used a Jeffery's hyperprior on each p_i , which is $\Pr(p_i) \sim \text{beta}(\alpha = 0.5, \beta = 0.5)$. We estimated the joint posterior probability of the genotypes and allele frequencies as $\Pr(g, p | x, \epsilon) \propto \Pr(x | g, \epsilon) \Pr(g | p) \Pr(p)$. Then we used Markov chain Monte Carlo (MCMC) described by Gompert et al. (2015) to estimate g (genotypes) and p (allele frequencies) posterior probability distributions. We ran 15,000 MCMC steps with a burn-in of 6,000 iterations with three chains. Minor allele frequencies (> 0.05) across all treatments were determined to indicate the frequency of the second most common allele or rare allele.

Data analysis

We quantified turnover of individuals under drought and irrigation treatments. The greater the individual turnover, the greater the potential for genetic differentiation among treatments. From the start of the experiment, we have been tracking the number of individuals to monitor survivors and new recruits. We do this using a computer algorithm which identifies survivors and new recruits based on their spatial locations in each quadrat (Lauenroth and Adler 2008).

Our objective for genetic diversity analyses was to determine how experimental (drought and irrigation treatments) and elevation (low and high elevation treatments) precipitation regimes affect overall levels of genetic variation. We used expected heterozygosity to measure the level of genetic variation across all treatments. Expected heterozygosity was calculated from allele frequencies (p_i) at the i^{th} SNP across individuals:

$$H_e = 2p_i(1 - p_i).$$

The value obtained ranges from 0 to 1, where a low value suggests low genetic variation.

We then used Sewell Wright's fixation index, F_{st} (Wright 1965), to partition genetic variation to determine if there is more genetic variation within or among treatments. We aggregated drought and irrigation treatments as the experimental precipitation regimes (drought vs. irrigation, D*I) and aggregated low and high elevation treatments (high elevation vs. low elevation, H*L) as the elevation precipitation regimes. We excluded the control treatment because it contained more genetic variability than the other treatments, which complicated the paired design. F_{st} was calculated from the total expected heterozygosity across all treatments (π_T) and from the average expected heterozygosity for each treatment (π_s):

$$F_{ST} = \frac{\pi_T - \pi_s}{\pi_T}.$$

The F_{st} value obtained ranges from 0 to 1, where a value <0.5 suggests there is stronger genetic differentiation within treatments. We then evaluated F_{st} across all SNPs using the same equation to compare genetic differentiation between D*I and H*L. SNPs found in the long tails of the F_{st} distribution would suggest that there are some SNPs undergoing

divergent selection. We compared D^*I and H^*L to examine the range of F_{st} across all loci, which helped us gauge which individual SNPs have high differentiation.

Lastly, we compared the F_{st} distribution of D^*I against H^*L to identify the individual SNPs that were associated between the experimental and elevation treatments. We quantified the proportion of SNPs found in the top 95th-98.5th percent quantile range to determine if there is an excess of SNPs that overlapped than what would be expected under null conditions. An excess of SNPs associated between the experimental and elevation treatments would suggest that there is consistency of selection and therefore evidence of individual SNPs undergoing selection. We determined the proportion of excess SNPs using a randomization test that generated null expectations with 10,000 iterations and quantified the excess of SNPs with an x-fold enrichment value. We calculated the x-fold enrichment by taking the observed number of SNPs and dividing by the mean of the null. For instance, an x-fold enrichment of 2.0 indicates that there are twice as many SNPs associated between the experimental and elevation precipitation regimes than expected by chance.

TABLE 1. Information about the number of *P. spicata* plant individuals collected from all precipitation treatments at USSES June 2016.

Treatment	Number of plant individuals
Control	66
Drought	103
Irrigation	62
High elevation	100
Low elevation	99

RESULTS

Effects of drought and irrigation on turnover of individuals

The number of individuals varied across the precipitation experiment treatments from the start of the experiment in 2011 through 2016. The number of surviving individuals was lower in the drought treatment than the irrigation treatment (Table 2). Over the course of the experiment, more recruitment occurred in the drought than in the irrigation quadrats.

Genetic variation between precipitation treatments

A total of 21,581 bi-allelic SNPs was identified across all plant individuals for all downstream genetic diversity analyses. We then estimated genotype and allele frequencies using a hierarchical Bayesian model. The minor allele frequency (MAF) distribution of all populations showed that there was a low frequency of the second most common allele (Fig. 1).

Our precipitation manipulations appeared to have no effect on the level of genetic variation. Genetic variation was similar between drought and irrigation (drought $H_e = 0.1088$, irrigation $H_e = 0.1087$) where the 95% confidence intervals overlapped and the expected heterozygosity was low (Fig. 2). These results indicate that the short-term changes to water availability did not affect overall levels of genetic variation.

In the high and low elevation treatments from the natural precipitation gradients, there was little difference in the level of genetic variation. The low elevation site had lower levels of genetic variation than the high elevation site (low elevation $H_e = 0.0931$, high elevation $H_e = 0.100$), but the expected heterozygosity was low for both treatments.

Even though there were minor changes in the level of genetic variation, the 95% confidence intervals overlapped, suggesting there were not any differences in overall levels of genetic variation between the low and high elevation populations (Fig. 2).

Genetic differentiation between precipitation treatments and across all loci

Overall F_{st} for D*I was low ($F_{ST} = 0.0129$), suggesting that there was little genetic differentiation among the experimental treatments. F_{st} for H*L was low ($F_{ST} = 0.0133$), which also suggests that there was little genetic differentiation.

When comparing F_{st} across all loci for both the experimental and elevation treatments, we examined the long tails of the F_{st} density curves for D*I against H*L. H*L had a longer tail, which suggests that there is more genetic differentiation of loci between the high and low elevation sites than loci in the experimental treatments (Fig. 3). This may indicate that the loci found in the long tails of H*L have stronger differentiation and thus could reflect divergent selection.

We then quantified the overlap of the long tails of the F_{st} density curves for D*I against H*L to find the proportion of the SNPs found across the empirical quantile range 95-98.5% that were associated between the experimental and elevation treatments. Overall, the x-fold enrichments for the shared top SNPs had twice as many shared SNPs than what would be expected under null expectations (Table 3, Fig. 4). For example, at the 95.5% quantile, we found 88 SNPs that were significantly associated between the experimental and elevation treatments (x-fold enrichment = 2.01, P-value < 0.01). This provides some evidence for evolutionary change where natural selection affected plant populations from both the manipulated and natural precipitation treatments in a consistent

way.

TABLE 2. Number of genets and genet turnover from the start of the precipitation experiment in 2011 through 2016.

Treatment	Number of individuals			
	Year 2011	Year 2016	Survivors	New recruits
Drought	158	154	37	117
Irrigation	195	106	41	65

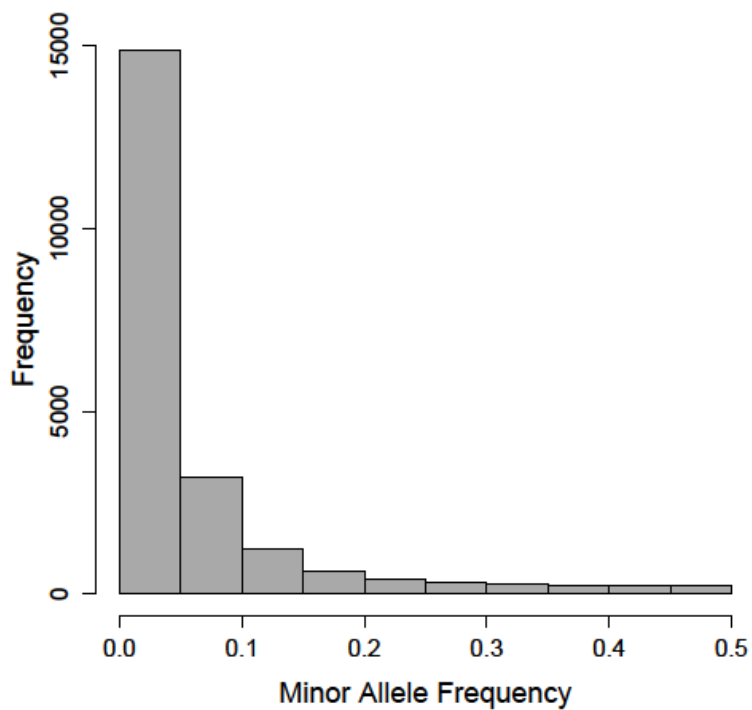


FIG. 1. Minor allele frequency across all precipitation treatments. Minor allele frequencies (> 0.05) across all treatments were determined to indicate the frequency of the second most common allele or rare allele.

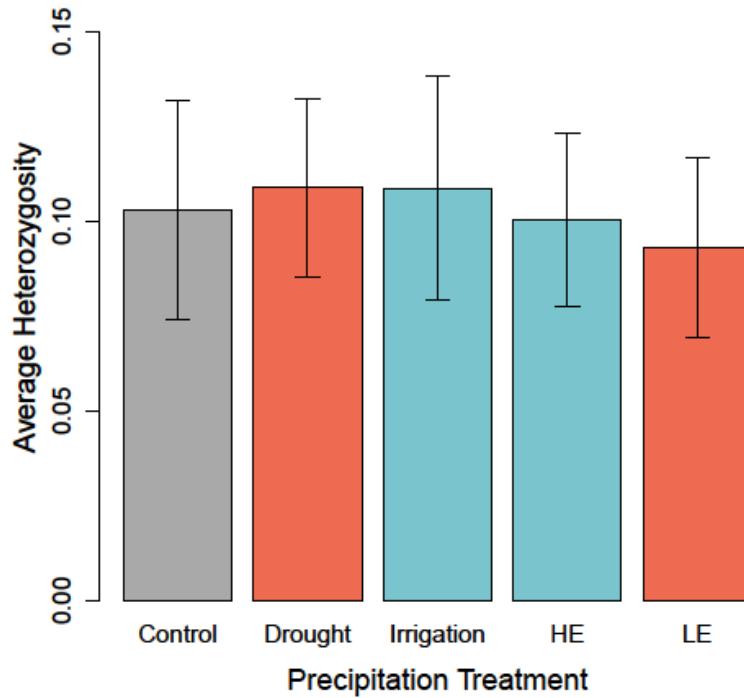


FIG. 2. The average expected heterozygosity with 95% confidence intervals of all precipitation treatments. Genetic variation was low and similar between drought and irrigation. There was more genetic variation in the high elevation site, but this was low, and the 95% confidence intervals lapped indicating that there were no true differences.

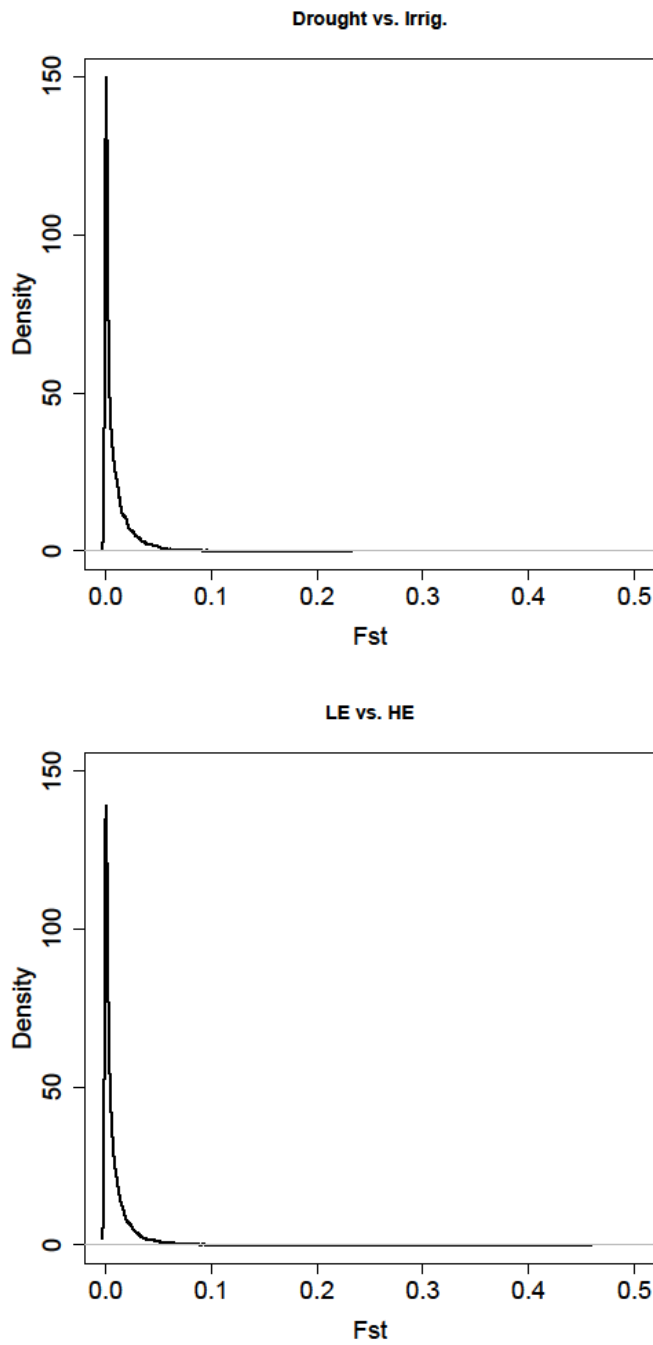


FIG. 3. F_{st} distributions across all loci among the experimental (top) and elevation precipitation treatments (bottom). When examining the long tails of both F_{st} density curves, the elevation comparison had a longer tail. This suggests that there was stronger genetic differentiation between populations at different elevations than between the experimental precipitation treatments.

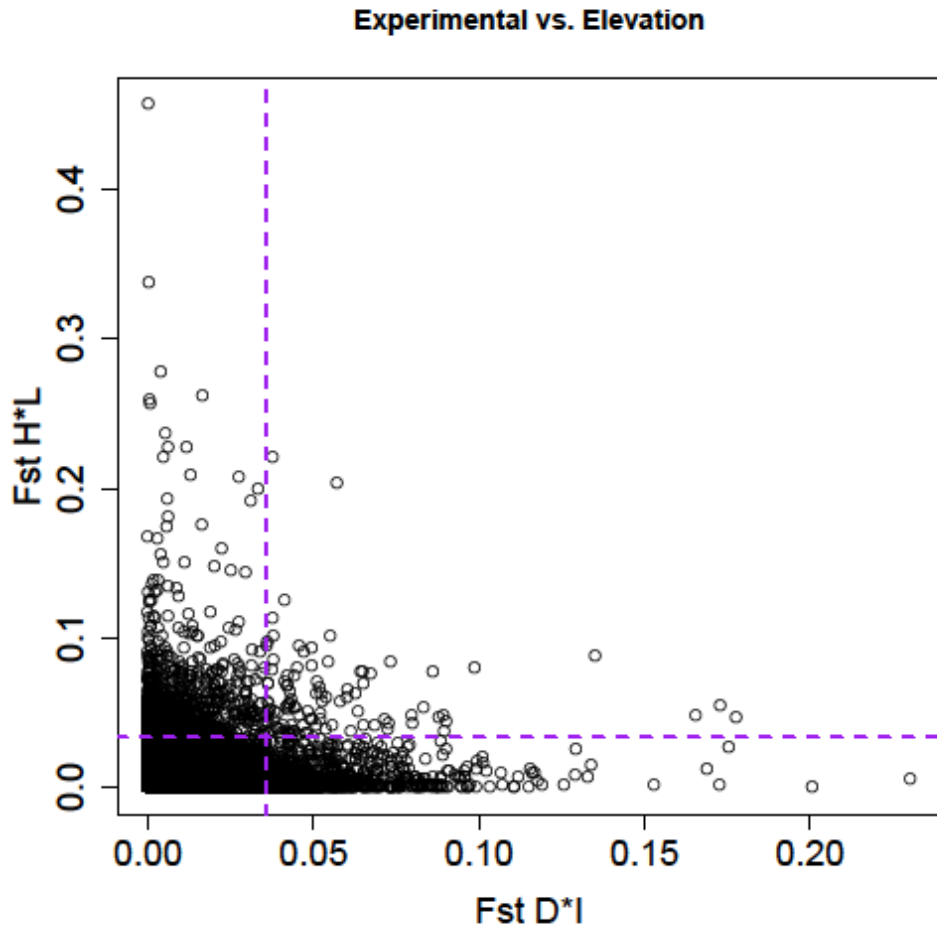


FIG. 4. F_{st} of the experimental (drought and irrigation, D*I) treatments against F_{st} of the elevation (high and low elevation, H*L) treatments. The dotted purple line is the 95th percentile quantile. There is a shared overlap of SNPs between the experimental and elevation treatments.

TABLE 3. Table showing the 95-98.5th percentile range for the observed shared overlap of the SNPs between the experimental and elevation precipitation treatments.

% Quantile	Observed SNPs	x-fold enrichment	P-value
95	105	1.94	< 0.01
95.5	88	2.01	< 0.01
96	71	2.05	< 0.01
96.5	62	2.34	< 0.01
97	44	2.60	< 0.01
97.5	30	2.23	< 0.01
98	23	2.66	< 0.01
98.5	12	2.46	< 0.01

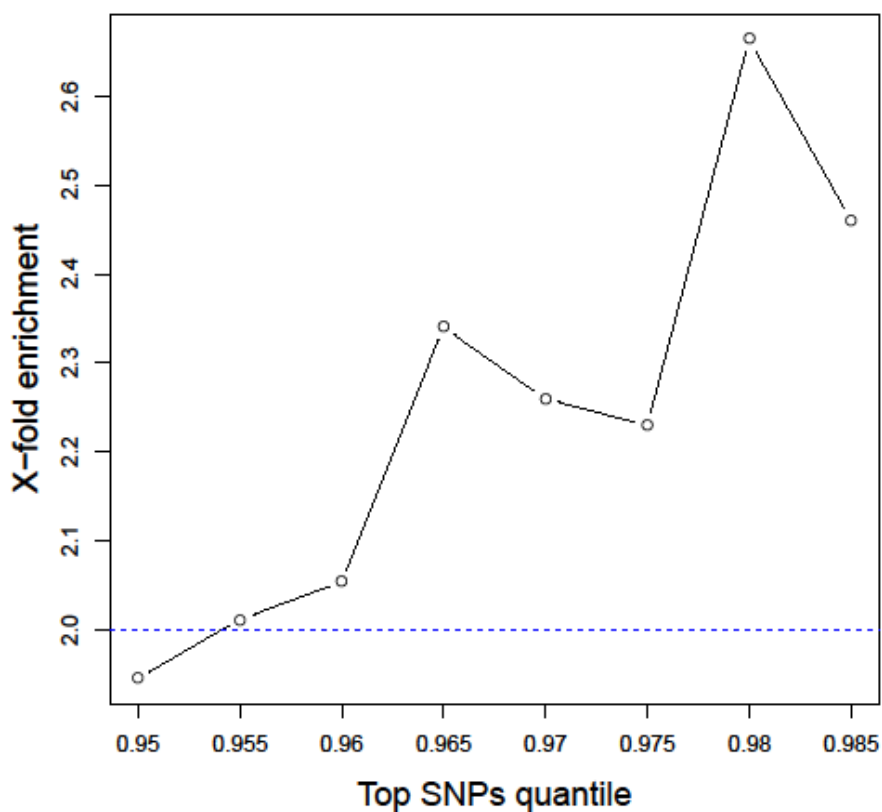


FIG. 5. Line plot showing the x-fold enrichments across the 95-98.5th percentile range for the shared overlap of SNPs between the experimental and elevation precipitation treatments. There was a significant excess of associated SNPs ($p < 0.01$) with ~ 1.94 - 2.66 times more than what is expected under null expectations.

DISCUSSION

Experimental precipitation reduction and addition appeared to have a subtle effect on demography and a weak effect on genetic variation. Our manipulated precipitation experiment showed that water availability does affect species turnover (Table 2), but there was no difference in overall levels of genetic variation (Fig. 2). However, examining genetic differentiation across all loci to compare experimental (D*I) versus elevation (H*L) treatments, we found some evidence of natural selection, which was consistent with spatial patterns along our natural precipitation regimes (Fig. 3 and 4).

Effects of drought and irrigation on species turnover and genetic variation

Overall, we found a weak genetic response, but our results were consistent with previous studies that showed *P. spicata* populations had weak demographic responses to our drought and irrigation treatments (Kleinhesselink 2017, Tredennick et al. 2018). These studies found that reduced water availability had a weak effect on plant basal cover, demography, and on the effect of soil moisture on aboveground net primary production. Despite moderate turnover among individuals, especially in the drought treatment where recruitment was higher, the expected heterozygosity between drought and irrigation was similar.

Why were levels of genetic variation similar between drought and irrigation? There are two possibilities for finding a weak response at the treatment level. First, *P. spicata* may be locally adapted to an arid environment by having a later growing season and a deep rooting system, which may explain why this bunchgrass is insensitive to variation in water availability (Munson and Long 2017). Also, in previous studies,

temperature had a stronger effect on plant populations than precipitation (Munson and Long 2017, Jordan et al. 2017), which may explain why we could not capture changes in allele frequencies at the population level.

Second, our manipulated precipitation experiment may not have induced wet and dry conditions extreme enough to push water availability outside the historical range (Kleinhesselink 2017, Tredennick et al. 2018). Kleinhesselink 2017 calculated the average seasonal soil moisture for our drought and irrigation treatments during each year of our experiment (2011 to 2016) and compared them to the 5th and 95th percentile quantile limits in the historical range (1929 to 2010). There were some dry and wet years throughout our experiment, but only the drought treatment reached extreme dry conditions, and this occurred only during one spring. In addition, the precipitation experiment may not have had enough time to induce environmental changes strong enough to cause changes in overall levels of genetic variation.

Comparison of genetic differentiation on experimental and natural precipitation regimes

We complemented our precipitation experiment with an ambient natural precipitation gradient to determine if patterns of genetic diversity under controlled precipitation could reflect what we see in nature. When comparing the natural precipitation gradient to our experimental treatments, we found subtle effects of genetic diversity, where there was slightly more genetic variation in the high elevation site. However, the overall levels of genetic variation for both elevations were low.

Regardless of finding similar levels of genetic variation between our experimental and elevation treatments, we partitioned genetic variation using F_{st} to determine how our

treatments were genetically differentiated. At the treatment level, F_{st} was low for both D*I and H*L, but at the loci level there was stronger differentiation between H*L. When examining SNPs in the tail of the F_{st} distribution between the experimental and elevation treatments, we found a significant excess of ~1.94-2.66 times more than what is expected under null expectations. Our results show that there is evidence of evolutionary change and suggests that the short-term changes in the experimental treatments were slightly consistent with differences found between the lower and higher elevation sites. We can conclude that precipitation did affect genetic differentiation for some loci.

Should ecological models include adaptive evolution?

Our results suggest that our current ecological models of *P. spicata* plant system may need to incorporate evolutionary adaptation. We did detect some evidence of natural selection and this was consistent under experimental and natural precipitation regimes, but we still do not know the population consequences. Evaluating the evolutionary consequences at the loci level alone does not provide enough information to justify the effort of incorporating adaptive evolution into our population models. Additional information is needed. First, showing that there is some selection operating may not provide enough information, we need to quantify the strength of selection. Second, we need to determine how variation in the population affects fitness. From our study, we know that climate can affect genetic diversity, but how does this in turn affect plant performance? Our study is a start to understand the evolutionary consequences to climate and continued research in ecological dynamics should consider the role of adaptive evolution in shaping populations.

CONCLUSION

In a fast-changing climate, forecasting population responses to global change is still an ongoing challenge and understanding how populations will persist is needed for mitigation strategies. We demonstrated how to use a population genomics approach to study how plant populations respond to experimental and natural precipitation regimes. Our study may provide a framework for future studies to understand the evolutionary consequences of climate change.

LITERATURE CITED

- Adler, P. B., K. M. Byrne, and J. Leiker. 2013. Can the past predict the future? Experimental tests of historically based population models. *Global Change Biology* 19:1793–1803.
- Adler, P. B., H. J. Dalglish, and S. P. Ellner. 2012. Forecasting plant community impacts of climate variability and change: when do competitive interactions matter? *Journal of Ecology* 100:478–487.
- Adler P.B., HilleRisLambers J., and Levine J. M. 2009. Weak effect of climate variability on coexistence in a sagebrush steppe community. *Ecology* 90:3303–3312.
- Anderson, J. T., J. H. Willis, and T. Mitchell-Olds. 2011. Evolutionary genetics of plant adaptation. *Trends in Genetics* 27:258–266.
- Barrett, R. D. H., S. M. Rogers, and D. Schluter. 2008. Natural selection on a major armor gene in threespine stickleback. *Science (New York, N.Y.)* 322:255–257.
- Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. *Trends in Ecology & Evolution* 23:38–44.
- Bell, G. 2017. Evolutionary Rescue. *Annual Review of Ecology, Evolution, and Systematics* 48:605–627.
- Bell, G., and A. Gonzalez. 2009. Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters* 12:942–948.
- Bell, G., and A. Gonzalez. 2011. Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. *Science (New York, N.Y.)* 332:1327–1330.

- Blaisdell, J. P. 1958. Seasonal Development and Yield of Native Plants on the Upper Snake River Plains and their Relation to Certain Climatic Factors. United States Department of Agriculture, Economic Research Service.
- Carroll, S. P., A. P. Hendry, D. N. Reznick, and C. W. Fox. 2007. Evolution on ecological time-scales. *Functional Ecology* 21:387–393.
- Chu, C., A. R. Kleinhesselink, K. M. Havstad, M. P. McClaran, D. P. Peters, L. T. Vermeire, H. Wei, and P. B. Adler. 2016. Direct effects dominate responses to climate perturbations in grassland plant communities. *Nature Communications* 7:11766.
- Coulson, T., B. E. Kendall, J. Barthold, F. Plard, S. Schindler, A. Ozgul, and J.-M. Gaillard. 2017. Modeling Adaptive and Nonadaptive Responses of Populations to Environmental Change. *The American Naturalist* 190:313–336.
- Dalgleish, H. J., D. N. Koons, M. B. Hooten, C. A. Moffet, and P. B. Adler. 2011. Climate influences the demography of three dominant sagebrush steppe plants. *Ecology* 92:75–85.
- Ehrlén, J., and W. F. Morris. 2015. Predicting changes in the distribution and abundance of species under environmental change. *Ecology Letters* 18:303–314.
- Franks, S. J., N. C. Kane, N. B. O’Hara, S. Tittes, and J. S. Rest. 2016. Rapid genome-wide evolution in *Brassica rapa* populations following drought revealed by sequencing of ancestral and descendant gene pools. *Molecular Ecology* 25:3622–3631.
- Fu, L., B. Niu, Z. Zhu, S. Wu, and W. Li. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* 28:3150–3152.

- Fussmann, G. F., M. Loreau, and P. A. Abrams. 2007. Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology* 21:465–477.
- Gompert, Z., A. A. Comeault, T. E. Farkas, J. L. Feder, T. L. Parchman, C. A. Buerkle, and P. Nosil. 2014a. Experimental evidence for ecological selection on genome variation in the wild. *Ecology Letters* 17:369–379.
- Gompert, Z., J. P. Jahner, C. F. Scholl, J. S. Wilson, L. K. Lucas, V. Soria-Carrasco, J. A. Fordyce, C. C. Nice, C. A. Buerkle, and M. L. Forister. 2015. The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation. *Molecular Ecology* 24:2777–2793.
- Gompert, Z., L. K. Lucas, C. A. Buerkle, M. L. Forister, J. A. Fordyce, and C. C. Nice. 2014b. Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. *Molecular Ecology* 23:4555–4573.
- Gompert, Z., L. K. Lucas, C. C. Nice, J. A. Fordyce, M. L. Forister, and C. A. Buerkle. 2012. Genomic Regions with a History of Divergent Selection Affect Fitness of Hybrids Between Two Butterfly Species. *Evolution* 66:2167–2181.
- Gomulkiewicz, R., and R. D. Holt. 1995. WHEN DOES EVOLUTION BY NATURAL SELECTION PREVENT EXTINCTION? *Evolution; International Journal of Organic Evolution* 49:201–207.
- Gomulkiewicz, R., S. M. Krone, and C. H. Remien. 2017. Evolution and the duration of a doomed population. *Evolutionary Applications* 10:471–484.
- Hendry, A. P. 2016. *Eco-evolutionary Dynamics*. Princeton University Press.
- Hermisson, J., and P. S. Pennings. 2005. Soft Sweeps. *Genetics* 169:2335–2352.

- Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485.
- Jordan, R., A. A. Hoffmann, S. K. Dillon, and S. M. Prober. 2017. Evidence of genomic adaptation to climate in *Eucalyptus microcarpa*: Implications for adaptive potential to projected climate change. *Molecular Ecology* 26:6002–6020.
- Josephs, E. B., J. R. Stinchcombe, and S. I. Wright. 2017. What can genome-wide association studies tell us about the evolutionary forces maintaining genetic variation for quantitative traits? *New Phytologist* 214:21–33.
- Kleinhesselink, A. 2017. Direct and Indirect Effects of Climate Change on Plant Populations and Communities in Sagebrush Steppe. All Graduate Theses and Dissertations.
- Kleinhesselink, A. R., and P. B. Adler. 2018. The response of big sagebrush (*Artemisia tridentata*) to interannual climate variation changes across its range. *Ecology*.
- Lafontaine, G. de, J. D. Napier, R. J. Petit, and F. S. Hu. 2018. Invoking adaptation to decipher the genetic legacy of past climate change. *Ecology* 99:1530–1546.
- Lande, R., and S. Shannon. 1996. The Role of Genetic Variation in Adaptation and Population Persistence in a Changing Environment. *Evolution* 50:434–437.
- Lauenroth, W. K., and P. B. Adler. 2008. Demography of perennial grassland plants: survival, life expectancy and life span. *Journal of Ecology* 96:1023–1032.
- Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27:2987–2993.
- Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows–

- Wheeler transform. *Bioinformatics* 25:1754–1760.
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079.
- Lindsey, H. A., J. Gallie, S. Taylor, and B. Kerr. 2013. Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature* 494:463–467.
- Munson, S. M., and A. L. Long. 2017. Climate drives shifts in grass reproductive phenology across the western USA. *The New Phytologist* 213:1945–1955.
- Orr, H. A. 2009. Fitness and its role in evolutionary genetics. *Nature Reviews Genetics* 10:531–539.
- Orr, H. A., and R. L. Unckless. 2008. Population extinction and the genetics of adaptation. *The American Naturalist* 172:160–169.
- Orr, H. A., and R. L. Unckless. 2014. The Population Genetics of Evolutionary Rescue. *PLOS Genetics* 10:e1004551.
- Parchman, T. L., Z. Gompert, J. Mudge, F. D. Schilkey, C. W. Benkman, and C. A. Buerkle. 2012. Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology* 21:2991–3005.
- Pelletier, F., D. Garant, and A. P. Hendry. 2009. Eco-evolutionary dynamics. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:1483–1489.
- Savolainen, O., M. Lascoux, and J. Merilä. 2013. Ecological genomics of local adaptation. *Nature Reviews Genetics* 14:807–820.

Tirmenstein, D. 1999. Species: *Artemisia tripartita*.

<https://www.fs.fed.us/database/feis/plants/shrub/arttrp/all.html>.

Tredennick, A. T., A. R. Kleinhesselink, J. B. Taylor, and P. B. Adler. 2018. Ecosystem functional response across precipitation extremes in a sagebrush steppe. *PeerJ* 6:e4485.

Wadgymar, S. M., D. B. Lowry, B. A. Gould, C. N. Byron, R. M. Mactavish, and J. T. Anderson. 2017. Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation. *Methods in Ecology and Evolution* 8:738–749.

Wright, S. 1965. The Interpretation of Population Structure by F-Statistics with Special Regard to Systems of Mating. *Evolution* 19:395–420.