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GENETIC DIFFERENTIATION OF TWO SPECIES OF BUCKWHEAT
(ERIOGONUM)

by

Jenessa Blotter Lemon

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

of

MASTER OF SCIENCE

in

Biology

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2017

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ABSTRACT

Genetic differentiation of two species of buckwheat (*Eriogonum*).

by

Jenessa B. Lemon, Master of Science

Utah State University, 2017

Major Professor: Dr. Paul G. Wolf
Department: Biology

Species delimitation is complicated by both biological and anthropological factors. Many species concepts have been proposed, but no one concept alone can account for all diversity found on the earth. Some species concepts cannot be applied to certain situations, and all species concepts fail when diverging taxa are observed while in the process of speciation. Circumscribing plant species is especially difficult because of their flexibility in hybridization. Complicated relationships with close relatives blur the boundaries between diverging plant species.

Discovering the extent of genetic differentiation between closely related taxa facilitates decisions regarding species protection under the Endangered Species Act. Here, we analyze genotype data to explore the relatedness of two buckwheat species: *Eriogonum soledium* - a narrow endemic under consideration for protection, and a widespread close relative, *Eriogonum shockleyi*. *Eriogonum soledium* grows only on Ordovician limestone outcroppings in west central Utah. The range of *E. shockleyi* is broad, spanning the western United States from Colorado to California, and Idaho to Arizona. *Eriogonum shockleyi* is suspected of hybridizing with other buckweats throughout this range. We found the genome of *E. shockleyi* to be rich with genetic diversity. In contrast, we found low levels of nucleotide diversity and estimated heterozygosity in *E. soledium*. One population, with genomic composition identifying with populations of *E. shockleyi*, was found

growing on Ordovician limestone, and morphologically identified as *E. soledium*. We hypothesize that phenotypic plasticity, edaphic adaptation, or both could cause *E. shockleyi* to appear even more similar to its close relative when grown on Ordovician limestone. We found moderate levels of divergence between the two taxa. The level of divergence suggests that these two species fall closer to the genetic divergence end of the continuum between no genetic distinction and complete genetic divergence. Based on these results, continued treatment of *E. soledium* as distinct from *E. shockleyi* is warranted.

(59 pages)

PUBLIC ABSTRACT

Genetic differentiation of two species of *Eriogonum*.

Jenessa B. Lemon

Limestone mining in the San Francisco Mountain Range of west central Utah threatens the survival of a rare endemic species of buckwheat (*Eriogonum soredium*). This species is an edaphic endemic, only found growing on the outcrops of the Ordovician limestone mines in the area. *Eriogonum soredium* is a candidate for governmental protection under the Endangered Species Act (ESA). However, a common, widespread buckwheat (*Eriogonum shockleyi*) appears to be closely related to the narrow endemic. The genetic relatedness of the rare and common species will greatly influence the decision of United States Fish and Wildlife Service (USFW) of whether or not to list the rare species for governmental protection. This study investigated the amount of genetic divergence between the two species to facilitate the decision. I found levels of population divergence intermediate between a state of no genetic distinction, and complete genetic divergence. However, the two species fall near the genetic divergence end of the continuum. This situation is not uncommon in plants, and suggests that the two species are currently in the process of speciation. Considering their morphological differences, and the ability of the genus *Eriogonum* to hybridize, these two species show significant amounts of divergence. These results suggest that the continued treatment of *E. soredium* as distinct from *E. shockleyi* is warranted. The USFW will use the results of this study to aid their decision of whether or not to list *E. soredium* under the ESA. Should the species be listed for protection under the ESA, limitations to the expansion of limestone mining in the San Francisco Mountain Range will be considered.

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Jenessa B. Lemon

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CHAPTER 1

INTRODUCTION

Species Delimitation of Rare Plants

It is difficult to deny the importance of defining species. In a broad sense, species are considered to be one of the fundamental units of biology (Mayr, 1982). Just as biological importance exists at the atomic, cellular, organismal, and population level, it is found at the species level. Beyond the desire to classify diversity, species identification has anthropological impacts. Species definitions determine the allocation of government time and resources. In 1979, the Endangered Species Act was put into place to protect species at risk of extinction in order to preserve their “esthetic, ecological, educational, historical, recreational, and scientific value to the Nation.” However, in order for a species to be protected under this legislation, it must first be defined.

Species Delimitation

Species delimitation is, and has always been, a highly-debated topic in biology (Mayden, 1999; Avise, 2000; De Queiroz, 2005; Rieseberg, Wood, and Baack, 2006; De Queiroz, 2007; Ellis, 2011). It is human nature for scientists to yearn for an all-encompassing, operational species concept that works for all organisms. However, because of the vast diversity found on earth, no single definition can accomplish such a goal. Many solutions to this problem have been proposed. Mayden (1999) elucidates at least 24 species concepts. However, most of these concepts can be grouped into several

categories, including: reproductive (biological, isolation, recognition), ecological, evolutionary, phylogenetic (Hennigan, monophyletic, genealogical, diagnosable), phenetic, and genotype cluster (Mayden, 1999). The first and famous biological species concept is known for its simplicity. Under the biological species concepts, species are reproductively isolated, interbreeding entities (Mayr, 1963). This concept is concise, and based on observable mechanisms. However, it is difficult to apply the biological species concept to extinct, cryptic, asexual, and other organisms for which we cannot observe reproduction. The beloved biological species concept also falls apart when applied to bacteria. In addition to asexual reproduction, bacteria cannot be evaluated with this concept because of horizontal gene transfer (Lawrence and Ochman, 1998). This concept is primarily used in animals, because of their lack of flexibility in reproduction. Under the ecological species concept, a species is a lineage, with its own ecological niche, on an independent evolutionary trajectory (Van Valen, 1976). Although this concept incorporates environmental factors, its criteria is flawed in that niches are difficult to define, and populations will often adapt to new niches as resource availability changes. The evolutionary species concept (Simpson, 1951) requires that a lineage evolves separately from others, with its own evolutionary aspects and trajectory. The phylogenetic species concept defines species as a basal clusters of organisms within which there is a “parental pattern of ancestry and descent” (Cracraft, 1983). Mayr (1942) posits a species concept based on isolation, in which species are groups of populations incapable of exchanging genes with other groups of populations because of reproductive isolation. The phenetic (or morphological) species concept, arguably even more simple than

biological species concept, distinguishes species by their phenotypic appearance (Shull, 1923). Although visibly classifying organisms might seem enticingly simple, this species concept has many flaws. The phenetic species concept is not ideal in situations of sexual dimorphism, phenotypic plasticity, and when working with extinct species.

In many circumstances, any one of these species concepts alone is not sufficient to define a new species. With numerous species concepts floating around, and heated debates surrounding the topic, Mayden (1999) proposed a hierarchy of the concepts in an attempt to satisfy as many points of view as possibly. He proposed a system in which one overarching, non-operational species concept is supplemented by the rest of the species concepts. De Queiroz (2007) argued that a single species concept, or even a hierarchy is unnecessary, but rather scientists should apply as many of the existing species concepts as possible to their research. This “unified species concept” promotes the continued search for methods of species delimitation. Clearly, the best species concept (or combination of concepts) needs to be evaluated for each phylogenetic study. To decide which species concepts should be applied, it is important to consider the different mechanisms and driving forces of speciation.

The Process of Speciation

At the molecular level, there is debate over the actual, physical way that genomes diverge. Case studies regarding the topic are scattered in their methodology and findings, resulting in the overall lack of a unified theory (Roux et al., 2016). Neutral theory (Kimura, 1991) proposed that most

polymorphisms are not selected or nor against (Hedrick, 2011). As mutation generates variation in a genome, genetic drift removes it. This balance between mutation and drift determines the amount of genetic variation present in natural populations. Because of the stochastic nature of mutation and genetic drift, if populations are prevented from exchanging genetic information, eventually they will diverge down their own evolutionary trajectories (Lenski and Travisano, 1994; Riesch et al., 2017). Gene flow is a strong force of genetic adhesion acting on populations (Mayr, 1963; Futuyma, 1987; Roux et al., 2016). Consequently, genetic differences between allopatric populations, incipient species, and closely related species are often largely defined by the amount of hybridization among them (Noor and Bennett, 2009; Payseur and Rieseberg, 2016; Roux et al., 2016). Investigating patterns of gene flow at the molecular level is of great importance when evaluating speciation events (Roux et al., 2016). If diverging allopatric populations are brought back into physical contact, there are several possible outcomes. One possibility is that enough divergence has occurred to establish reproductive isolation, and differences persist between the populations. Another possibility is that reproductive barriers fail, allowing admixture to eventually homogenize the populations, and halting the speciation process. Another possibility is that hybrid individuals are created, intermediate between the two parent populations. The extent of hybridization between two species is often influenced by the fitness of hybrid individuals. In cases of hybrid vigor or "heterosis," hybrids have increased fitness, because of heterozygote advantage, and their ability to mask deleterious recessive alleles (Lynch, 1991; Edmands, 1999). In this situation, hybrids are selected

for. However, sometimes hybrid individuals interfere with local adaptation, and are selected against (Templeton, 1981). Hybrid fitness depression discourages gene flow between populations. Interruptions in gene flow between populations is one of the major driving forces of divergence.

Geographic distance is an obvious cause of the cessation of gene flow between populations. As a successful taxon extends its geographic range, populations lose contact because the distances become too great for dispersal and mating. In the case of adaptive radiation, in which an ancestral species undergoes rapid diversification to fill a variety of ecological niches, this process can happen quickly. In addition to random mutation, populations throughout extensive ranges are selected for by different environmental factors, and exposed to hybridization with different groups. Without genetic communication, distant populations diverge down their own evolutionary trajectories.

Sympatric Speciation

Although long distances can restrict gene flow, it is possible for divergence to occur sympatrically, even in the presence of gene flow. Sympatric speciation is made possible by the formation of internal reproductive barriers between groups (as opposed to geographic barriers). Natural selection can propagate divergence between sympatric populations through disruptive (or divergent) selection (Seehausen, 2004; Räsänen and Hendry, 2008; Nosil, Harmon, and Seehausen, 2009). Divergent selection targets areas of the genome that are concentrated with expressed genes, specifically those contributing to the establishment of barriers contributing to

reproductive isolation (Nosil et al., 2017). In the presence of reproductive barriers, divergence throughout the genome is promoted through genetic drift. Even neutral alleles may be affected by divergent selection through linkage relationships. In divergent hitchhiking, gene flow is additionally reduced in regions linked to loci under divergent selection (Powell et al., 2013). These circumstances may facilitate the maintenance of species during instances of secondary contact (Powell et al., 2013). Of course, in a broad sense, divergent selection promotes speciation by discouraging gene flow between populations. It is also possible that natural selection has a role much earlier in the speciation process. If populations evolve in parallel when exposed to similar environments, the source of the unity is likely natural selection (Rundle et al., 2000). Genetic drift is incapable of causing directed change in multiple lineages, and can be ruled out as the cause of this phenomenon. Parallel speciation results in reproductive isolation between populations that inhabit different environments, but compatibility between populations in similar habitats (Schluter and Nagel, 1995). Parallel speciation implies that natural selection can be an important factor driving speciation. Rundle et al. (2000) investigated the possibility of parallel speciation in threespine sticklebacks, and found a strong correlation between ecomorphs with niches in similar lake depths. Sticklebacks were no more likely to mate with members from their own lake than members from another lake, assuming all had a habitat the same lake depth. This study suggests that natural selection, in the form of divergent selection of key traits influencing reproduction, has a role to play in speciation. Evidence of parallel ecological speciation is observed less commonly in plants than animals, nevertheless there are many potential

examples (Ostevik et al., 2012). The scarcity of evidence in plants suggests that parallel speciation is less common, but could also be due to a lack of thorough research on the subject (Ostevik et al., 2012). In addition to the selective forces influencing sympatric speciation, support for the possibility of divergence under the influence of gene flow is also found at the molecular level.

According to the genic view of speciation, genomic “islands of divergence” develop while the rest of the genome remains under the influence of gene flow (Lexer and Widmer, 2008; Noor and Bennett, 2009; Hohenlohe et al., 2010; Feder, Egan, and Nosil, 2012; Martin et al., 2013). In the genic view, differentiation must hold up under incidences of secondary contact, and be incapable of sharing adaptive alleles (Lexer and Widmer, 2008). The genic view of speciation emerged from the results of many studies of closely related species showing high levels of divergence in regions of hybrid genomes with restricted recombination (Noor and Bennett, 2009). Chromosomal rearrangements, sex chromosomes, or regions near centromeres (where crossover events and gene conversion are less likely) are examples of such areas (Hoffmann and Rieseberg, 2008). Models of parapatric speciation corroborate this theory by suggesting that some kinds of incompatibility are more likely to develop in the presence of inversions (than areas with higher levels of recombination). Inversions may also promote speciation with gene flow by promoting linkage disequilibrium between genes associated with hybrid fitness depression, directional selection, and assortative mating (Butlin, 2005). Recombination hotspots are candidates for potential “islands” of divergence, and might be responsible for maintaining species during initial

separation and incidences of secondary contact (Hoffmann and Rieseberg, 2008; Noor and Bennett, 2009; Roux et al., 2016). However, some argue that much of the evidence gathered to investigate the topic may not be valid (Noor and Bennett, 2009). One strong piece of evidence that brings the “islands of diverge” model of speciation into question is the fact that regions with chromosomal rearrangement may show higher levels of differentiation in all cases, not just those regarding speciation events. If chromosomal rearrangements ubiquitously cause linkage disequilibrium, and only sometimes cause speciation events, we cannot assume that these areas are significantly promoting speciation. In addition, rearrangements that segregate within many species can reduce homogenization as soon as they appear. If these highly divergent rearrangements eventually fix, they can be wrongfully interpreted as situations of speciation with gene flow (Noor and Bennett, 2009). The effects of chromosomal rearrangements may also be biased because of the ability of regions to “hitchhike” along with them. As large chromosomal rearrangements disperse throughout the genome, they replace nucleotide polymorphisms, and temporarily reduce the level of variation. This process artificially inflates measures of relative divergence (Noor and Bennett, 2009). It is also important to remember that the genomes of plants and animals often behave differently (Grant, 1971; Wu, 2001). To date, most studies reporting islands of divergence in concordance with the genic view of speciation are animal studies (Wu, 2001). Further research is needed to determine whether some aspects of the the genic view of speciation are applicable to plants. However, key aspects of the thoery are observed in plants, such as the accumulation of reproductive barriers in genomic areas of

chromosomal rearrangement (Rieseberg, Whitton, and Gardner, 1999; Rieseberg, 2001). Furthermore, one study evaluated transcriptome scans among recently diverged sunflowers, and found the formation of genomic islands of divergence in regions with reduced rates of recombination (Renaut et al., 2013). Central to debate of the possibility of speciation under the influence of gene flow, is the concept of a speciation continuum.

A Speciation Continuum

Recently, research has been focused on speciation as process, rather than an event (Hendry et al., 2009). This shift was propelled by the “species continuum concept,” which refers to the consecutive genetic changes leading to divergence between lineages (Shaw and Mullen, 2014). The species continuum concept proposes that there exists a continuum between panmixis and reproductive isolation. Populations currently in the process of speciation can be found in several states: continuous variation (in situations of panmixis), some differentiated variation with reproductive barriers beginning to form, much differentiated variation with strong, but impermanent reproductive barriers, and complete and permanent reproductive isolation (Shaw and Mullen, 2014). However, these states are not abrupt, hence the term “continuum.” Species can move through the states sequentially, or jump straight from continuous variation (which is always the starting point) to permanent isolation. Species can even bounce back and forth between states in both directions. The idea is that a genome can exist in an intermediate state between panmixis and reproductive isolation. This intermediate state is made possible by the formation of incomplete reproductive barriers between groups.

Incomplete reproductive barriers between species limits gene flow, resulting in individuals with mixed genomic composition. It is not uncommon to uncover ecologically driven speciation events that never reach complete reproductive isolation (Nosil, Harmon, and Seehausen, 2009). In this situation, diverging taxa can be found in a gray zone of speciation (Roux et al., 2016). Generally, net synonymous divergence of at least 2% warrants the assignment of a new species (Avice, 2000; Hebert et al., 2003; Nielsen and Matz, 2006; Roux et al., 2016). However, Roux et al. (2016) discovered a gray zone of speciation between 0.5 - 2% net synonymous divergence. This study did not find species, life history, or ecology to affect this range. Determining relationships in this intermediate range is confusing and arguably inaccurate. One study investigated 36 genetic markers (35 nuclear and 1 mitochondrial) in the red backed fairy wren and found reproductive barriers beginning to form at low levels (.075%) of net synonymous divergence (Lee and Edwards, 2008). In addition, significant genetic separation is observed before phenotypic separation begins in some species, while in others, hybrid depression is observed early in the process.

A recent study (Nosil et al., 2017) suggests that one form of continuous speciation involves the gaining of mutations until they reach a “tipping point,” at which point major transformations are established through rapid change in the population (Nosil et al., 2017). This form of speciation is gradual, until the tipping point is reached, then adaptation occurs at a rapid rate. This method usually results in either a single species with little variation (before the tipping point is reached), or two differentiated species (after the tipping point is reached). Although gradual, this process is not necessarily slow, and can

happen rather quickly in situations of positive feedback between changes in allele frequencies (Räsänen and Hendry, 2008). Tipping points are present in other complex systems found in health, ecology, and economics (Nosil et al., 2017). Whether populations are susceptible to sudden shifts (tipping points) has to do with connectivity. Gene flow homogenizes populations, preventing adaptation. These complex systems exhibit warning signs when the situation is near its “tipping point.” High levels of variance, transitioning back and forth between the two stable states, and slow return from one state to the other following small shifts are examples of such warning signs (Nosil et al., 2017). Discovering biological warning signs that indicate impending rapid divergence between taxa would be a useful tool to be applied to conservation.

Although gradual speciation is frequently observed in nature, at least one common mechanism of speciation can be completed in a single generation. Polyploidy is a sympatric, saltational method of speciation. Polyploid species are the result of nondisjunction in meiosis and involve the duplication of an entire genome. The enormous difference in gene dosage between the parent and offspring can sometimes result in a reproductive barrier (Wendel, 2000). Vascular plants are primarily susceptible to these speciation events: 47-100% of flowering plant species can be traced back to a polyploid speciation event in evolutionary history (Masterson, 1994; Wood et al., 2009). In addition to polyploidization, rapid evolution can result from extremely rare cases such as founder events, and crucial mutations that confer reproductive isolation (Barton and Charlesworth, 1984). These near instantaneous speciation events provide clear boundaries, leaving less ambiguous advice for species delimitation.

The Genetics of Rare Plants

Professionals in all fields treasure rarity. It is no surprise that a large amount of resources are allocated to understanding the genetics of rare plants (Kruckeberg and Rabinowitz, 1985). Molecular attempts at species delimitation often involve at least one rare taxa. Small population sizes and restricted geographic ranges cause the populations of rare plant species to behave differently than common ones. Two main factors affecting the genetics of small populations are genetic drift and inbreeding depression (Allendorf, 1983; Falk and Holsinger, 1991; Lynch, 1991). Genetic drift refers to the change in the allele frequencies of a population passed down from one generation to the next, due to sampling effects caused by small numbers of mating individuals. Genetic drift reduces within population variation, and increases between population variation (Ellstrand and Elam, 1993). The sample size of alleles passed down from one generation to the next (or effective population size) can further be reduced because of overlapping generations, unequal numbers of reproducing male and female organisms, and rapid changes in population size (Franklin, 1980; Lande, 1988; Falk and Holsinger, 1991). Effective population size is usually lower than the total number of individuals in the populations by 0-75% (Nunney and Campbell, 1993). In situations of extremely low effective population size, such as bottleneck or founder events, significant changes in allele frequencies can be observed over a single generation (Wright, 1931; Barton and Charlesworth, 1984; Falk and Holsinger, 1991). Furthermore, Lynch and Gabriel (1990) illustrate a dangerous positive feedback loop in these situations that can lead

to “mutational meltdown.” As population size decreases, genetic drift can overpower selection, resulting in the fixation of deleterious alleles (Lynch and Gabriel, 1990). The resulting negative fitness effects cause continual reduction in population size. Population reduction inflates the impact of genetic drift, and the cycle continues, spiraling toward extinction (Lynch and Gabriel, 1990).

Small populations are also more susceptible to inbreeding depression (Allendorf, 1983; Lynch, 1991). Inbreeding results when related individuals reproduce. The most severe case of inbreeding is self fertilization. In small populations, there is a greater chance of biparental inbreeding because of the higher probability of relatedness between individuals. In situations where opportunities for outcrossing are rare, populations may adapt toward self fertilization to ensure an opportunity for reproduction (Ellstrand and Elam, 1993). A selfing plant has the potential to pass more of its genes to the next generation, because not only can they send off their own gametes to hopefully be fertilized, but they can fertilize their own, insuring at least some genetic transmission (Campbell, 2015). However, negative side effects come with this promise of reproduction. Inbreeding depression is characterized by high levels of homozygosity, resulting in the expression of deleterious recessive alleles (Barrett and Charlesworth, 1991; Lynch, 1991). If inbreeding persists, homozygosity increases, and alleles will eventually reach fixation (Wright, 1931; Lynch, 1991). This loss in heterozygosity is costly, leaving populations with less resilience. Nucleotide diversity allows for adaptation to environmental changes, and recovery from epidemics and environmental catastrophe (Charlesworth and Willis, 2009).

In addition to small population sizes, rare plants commonly have ecologically or spatially restricted ranges. Plants with ecologically restricted ranges have often diverged from a widespread progenitor (Kruckeberg, 1991; Safford, 2011). By adapting tolerance for a harsh environment, diverging endemics are able to survive in a distinct habitat with less competition (Stebbins and Major, 1965; Heydel et al., 2017). Because plants rely on the soil beneath them for water and nutrients, it is not surprising that edaphic qualities are a common environmental factor separating species in this way. Serpentine, limestone, granite, and acidic substrates are often rich with endemic species (Kruckeberg, 1991). However, endemic plants with narrow geographic ranges are at an increased risk of extinction by environmental changes or catastrophe along with the molecular consequences mentioned previously (Hamrick and Godt, 1990; Kruckeberg, 1991; Lande, 1993; Stebbins Jr, 2013).

In the next chapter, I investigate the genetic relatedness of two species of buckwheat (*Eriogonum*). *Eriogonum soledium* is endemic to the San Francisco Mountain range in west central Utah. This edaphic specialist grows only on Ordovician limestone outcrops, and is under consideration for protection under the ESA. The status of this species will be influenced by its relationship to a close relative, *Eriogonum shockleyi*. *Eriogonum shockleyi* is common throughout the western United States, with a broad geographic range spanning 8 states (including the range of *E. soledium*). I use genomic single nucleotide polymorphism data to measure the levels of nucleotide diversity and estimate heterozygosity in the two species. I explore the effects of isolation by distance on remote populations of *E. shockleyi*. I investigate

patterns of gene flow and signatures of admixture between the two species, and use insights from the data to make conclusions about their genetic relatedness. I consider edaphic endemism as a possible cause for the formation of incomplete reproductive barriers between the two species, and provide conservation recommendations to encourage the survival of *E. soledium*.

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CHAPTER 2

GENETIC DIFFERENTIATION OF TWO SPECIES OF BUCKWHEAT (*ERIOGONUM*)

Introduction

The Endangered Species Act (1973) aims to protect species at risk of extinction in order to preserve their “esthetic, ecological, educational, historical, recreational, and scientific value to the Nation.” However, ESA status can be ambiguous because of the difficult task of delimiting closely related taxa. Species boundaries are highly dependent on the species concepts used in delimitation. Ideally, there would be an all-encompassing, operational species concept that works for all organisms. However, because of the vastness of biological diversity, no single concept can accomplish this goal. Mayden (1999) reviews at least 24 species concepts, most of which can be grouped into: reproductive, ecological, evolutionary, phylogenetic, phenetic, and genotypic. In many circumstances, any one of these concepts alone is not sufficient to circumscribe species. Furthermore, all species concepts fail when two diverging taxa are observed in the process of speciation. Often however, molecular studies can be used to examine evolutionary histories, with hopes of untangling complex phylogenetic relationships, thereby enabling a functional recognition of taxa.

Neutral theory (Kimura, 1991) proposes that most polymorphisms are not selected for or against (Hedrick, 2011). As mutation generates variation in a genome, genetic drift removes it. This balance between mutation and drift determines the amount of genetic variation present in natural populations.

Because of the stochastic nature of mutation and genetic drift, if populations are prevented from exchanging genetic information, eventually they will diverge down their own evolutionary trajectories (Lenski and Travisano, 1994; Riesch et al., 2017). Gene flow is one of the major forces of genetic cohesion acting on populations (Mayr, 1970; Futuyma, 1987; Gompert et al., 2014; Roux et al., 2016). Consequently, genetic differences between taxa are often largely defined by the amount of hybridization between them (Noor and Bennett, 2009; Payseur and Rieseberg, 2016; Roux et al., 2016). Hybridization between diverging species leaves behind evidence of admixture and introgression, facilitating the efforts of scientists to hypothesize evolutionary history and delimit species. Species with broad ranges are susceptible to isolation by distance, which restricts gene flow between distant populations and allows differences introduced by mutation and hybridization to accumulate. Other biological processes complicate species delimitation. As plant species begin to diverge, reproductive isolation can initially be weak, allowing for stable hybrids with intermediate genome composition (Rieseberg and Willis, 2007). Recent or rapid diversification events, such as adaptive radiation, can also result in intermediate genomes, making species boundaries hard to define (Wendel and Doyle, 1998). Additionally, environmental effects on morphology are capable of masking or exaggerating the progress of genetic differentiation (Rajakaruna, 2004).

There are two extreme states of population divergence. In the first, there is no genetic distinction between populations. In the second, populations are genetically distinct, and fixation for different alleles has been reached in multiple genes. It is not uncommon to find natural populations that fall

somewhere between these two extremes of population divergence in plants. Plants tend to have flexibility in hybridization, allowing gene flow to persist even when a significant amount of genetic divergence has developed between populations. Furthermore, taxa that are currently in the process of speciation will also fall somewhere between the two extremes. With so much to consider at, and above, the molecular level, untangling the relationship between diverging taxa can be difficult. However, consideration for listing a species under the ESA requires a verdict as to whether two diverging groups are sufficiently distinct to be considered different taxa.

Under the ESA, species can be listed as threatened or endangered. These classifications are greatly influenced by population size. Smaller populations are more susceptible to extinction due to random local disturbances, and tend to have smaller effective population sizes (Schemske et al., 1994). In addition to size, population vital statistics (birth, growth, death, survivorship, and fecundity) and metapopulation factors (extinction and colonization rates) also affect rates of extinction (Schemske et al., 1994). If a species is listed as endangered, federal law protects not only the organism, but also the habitat on which the organism depends. The U.S. Fish and Wildlife Service is primarily responsible for enforcing the ESA for land and freshwater organisms. Because government intervention can affect the economic potential of privately owned land, ethical and accurate listing decisions are essential. Whether species are sufficiently distinct from closely related, widespread taxa to warrant protection under the ESA can be obscure, and necessitate genetic population analysis (Falk and Holsinger, 1991; Smith

and Bateman, 2002). Here we consider two species of *Eriogonum*, an extreme edaphic specialist, and a widespread, edaphic generalist.

Eriogonum is a genus of North American buckwheats, with over 250 species (Grady and Reveal, 2011; Grady, 2012) ranging from Alaska to central Mexico, and from the offshore islands of California to West Virginia (Reveal, 1978). This genus is known for rampant hybridization. Frisco Buckwheat, *E. soredium*, grows only on Ordovician Limestone shale and is endemic to Beaver and Millard counties in Utah (Grady and Reveal, 2011; Hildebrand, 2013). The range of *E. soredium* is less than eight square km, located in the San Francisco Mountain Range. Recruitment in these populations is low; juvenile plants and seedlings are only found in a few populations (Kass, 1992; Roth, 2010). The plant grows 2-4 cm tall, and 10-50 cm across (Welsh, 2008). The white (to light pink) flowers grow in clusters, and leaves are 2-5 mm long and covered in small white hairs (Welsh, 2008). Flowering occurs June-August. The total population of *E. soredium* is unknown, but the USFW estimates 78,500 surviving individuals (M. Wheeler, Utah Division of Natural Resources, personal communication, 2017).

Eriogonum soredium is a candidate for federal listing under the Endangered Species Act, however, the species appears to be closely related to Shockley's buckwheat, *E. shockleyi* (Grady, 2012). *Eriogonum shockleyi* has several varieties and is found throughout the western United States in California, Nevada, Idaho, Utah, New Mexico, Colorado, and Arizona. The two species look very similar, and have overlapping geographical ranges. Here, I used single nucleotide polymorphisms (SNPs) from RAD-seq analysis to examine genetic variation within and between these two species.

The main objectives of this project were 1. To examine the amount of, and patterns of, genetic variation in *E. soredium* and *E. shockleyi*. 2. To infer the genetic relatedness of *E. shockleyi* and *E. soredium*. 3. To determine if *E. soredium* is sufficiently distinct from *E. shockleyi* to warrant continued treatment as a separate species. 4. If *E. soredium* is found to be distinct, we will examine the degree to which it hybridizes with *E. shockleyi*.

MATERIALS AND METHODS

Sampling

The goal was to gather samples that represent the range and diversity of each species. Each collection site, here referred to as a population, represents a small group of geographically isolated and (presumably) interbreeding individuals. I sampled 118 individuals of *E. shockleyi* from 27 populations, including samples of *var. shockleyi*, *var. longilobum*, and *var. packardae* (Fig. 1, Table 1). I sampled 37 individuals from five populations of *E. soredium* using data from (Robinson, 2004) to find the populations (Fig. 2). I sampled from individuals more than 1 meter apart, in order to avoid resampling of the same plant twice. These species of *Eriogonum* are known to form clones up to about a meter (Welsh, 2008).

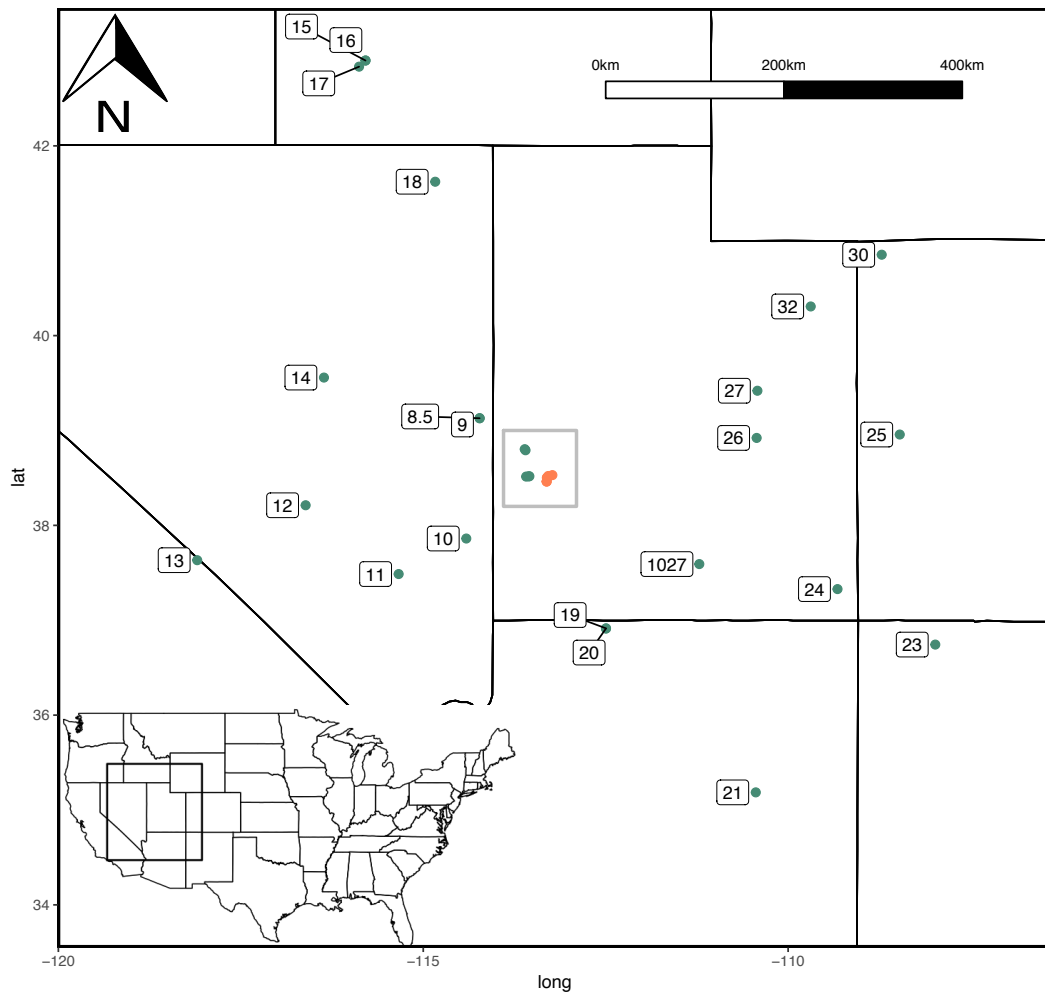


Figure 1. A map of the western United States, showing all sampling sites. *E. shockleyi* is shown in teal and *E. soredium* in shown in orange. The grey box encloses samples collected from the San Francisco Mountains.

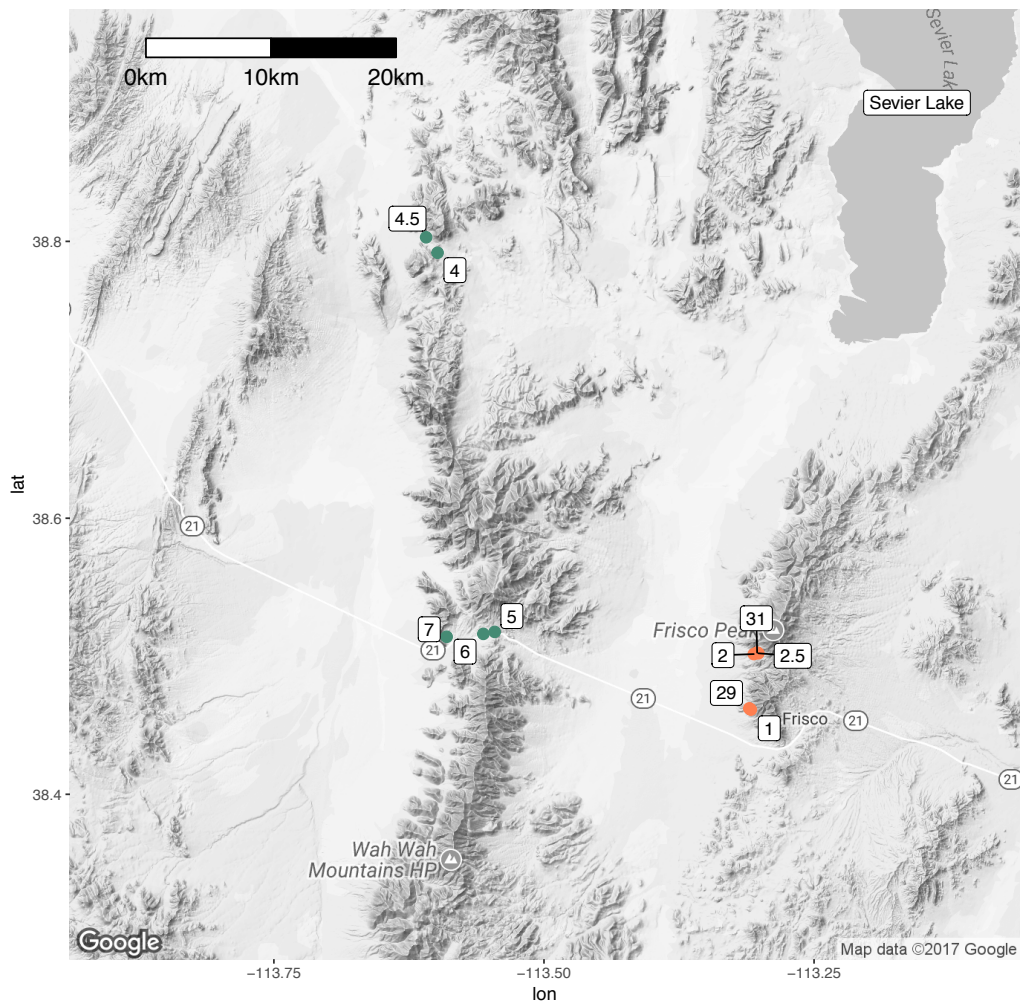


Figure 2. A map of sampling sites in the San Francisco Mountain Range. Populations of *E. shockleyi* are shown in teal and *E. soredium* are shown in orange.

Number	Taxon	lat long (deg)	Number of Samples
LEMON-001	<i>E. soledium</i>	38.4614 -113.3085	7
LEMON-002	<i>E. soledium</i>	38.5016 -113.3058	11
LEMON-002.5	<i>E. soledium</i>	38.5022 -113.3016	6
LEMON-029	<i>E. soledium</i>	38.4623 -113.31	6
LEMON-031	<i>E. soledium</i>	38.5027 -113.3027	7
LEMON-004	<i>E. shockleyi var. shockleyi</i>	38.7918 -113.5985	1
LEMON-004.5	<i>E. shockleyi var. shockleyi</i>	38.803 -113.6091	6
LEMON-005	<i>E. shockleyi var. shockleyi</i>	38.5177 -113.5456	5
LEMON-006	<i>E. shockleyi var. shockleyi</i>	38.5163 -113.5561	6
LEMON-007	<i>E. shockleyi var. shockleyi</i>	38.5142 -113.5901	6
LEMON-008.5	<i>E. shockleyi var. shockleyi</i>	39.1292 -114.2272	1
LEMON-009	<i>E. shockeyi</i>	39.1292 -114.2271	6
LEMON-010	<i>E. shockeyi</i>	37.8612 -114.4104	3
LEMON-011	<i>E. shockeyi</i>	37.4869 -115.3371	4
LEMON-012	<i>E. shockleyi var. shockleyi</i>	38.2119 -116.6116	5
LEMON-013	<i>E. shockeyi</i>	37.6329 -118.0975	5
LEMON-014	<i>E. shockleyi var. shockleyi</i>	39.5577 -116.3607	6
LEMON-015	<i>E. shockeyi</i>	42.8992 -115.791	3
LEMON-016	<i>E. shockeyi</i>	42.8999 -115.7912	8
LEMON-017	<i>E. shockleyi var. packardae</i>	42.8347 -115.8794	5
LEMON-018	<i>E. shockleyi var. packardae</i>	41.6219 -114.836	4
LEMON-019	<i>E. shockeyi</i>	36.9143 -112.4954	3
LEMON-020	<i>E. shockleyi var. longillobum</i>	36.9126 -112.4963	4
LEMON-021	<i>E. shockeyi</i>	35.1857 -110.4432	6
LEMON-023	<i>E. shockleyi</i>	36.7434 -107.9835	1
LEMON-024	<i>E. shockeyi</i>	37.3283 -109.3234	4
LEMON-025	<i>E. shockeyi</i>	38.9573 -108.4703	1
LEMON-026	<i>E. shockeyi</i>	38.9216 -110.4312	4
LEMON-027	<i>E. shockeyi</i>	39.4189 -110.421	5
LEMON-030	<i>E. shockeyi</i>	40.8526 -108.7176	7
LEMON-032	<i>E. shockeyi</i>	40.3072 -109.6904	1

WOLF-1027	<i>E. shockleyi</i> var. <i>shockleyi</i>	37.592 -111.2173	8
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Table 1. Summary of populations collected. Locality information for populations of *E. soredium* are not provided, because the species is under consideration for protection under the ESA.

Sampled leaf tissue was placed inside a small envelope or coffee filter, and dried rapidly on silica gel. Approximately 10 leaves of *E. shockleyi* and 20 leaves of *E. soredium* were collected from each sample. Plants were chosen randomly, but in a representative way for each population (plants were chosen in the center, borders, and areas in between from each population.) Samples were not selected based on physical appearance, but only plants with enough healthy tissue were selected in order to avoid inflicting lethal damage to the individual. I deposited two vouchers from each sampling site at the Intermountain herbarium (UTC). However, only one voucher was collected at some of the sites, because of the limited number of individuals present. Because federal protection of *E. soredium* is under consideration, locality information is not provided for populations of this species.

Data Acquisition

I extracted DNA from each plant using the Qiagen DNeasy 96 kit (Cat. No. 69181; Qiagen Inc., Valencia, CA, USA,) modifying the protocol slightly. Due to the tough nature of the desert plant, I soaked the dried leaf tissue in AP1 buffer for 30 minutes before tissue lysis, and kept samples in the tissuelyser for a longer period of time than specified in the protocol (3m for each rotation of the 96 well plate). Following DNA extraction and

normalization, I prepared genomic libraries using a restriction-site associated DNA sequencing (RAD-seq) double enzyme digest approach (Parchman et al., 2012; Gompert et al., 2014). Genomic DNA was cut with *MseI* and *EcoR1* restriction enzymes, and adaptors and internal Illumina index barcodes were added to track samples. Samples were pooled, and fragments were PCR amplified. Size selected DNA fragments (300-400 bp) were sequenced for 100 bp from one end using an Illumina HiSeq platform. I used the data assembly software *iPyRAD* (Eaton, 2014) to assemble the raw DNA sequence data into genotype formats for further analysis. *iPyRAD* first demultiplexes the raw data by barcode, and low quality base calls are filtered. Within-sample clusters are generated using *USEARCH* (Edgar, 2010), and reads are aligned using *MUSCLE* (Edgar, 2004). Error rate and heterozygosity are then estimated, and consensus bases are called and filtered. Finally, clusters are generated across samples, and filters are applied to the resulting data, generating a number of genotype output formats. Due to the lack of a reference genome, *iPyRAD* assembled the data *de novo* using *vsearch* (Enns, Ochs, and Rensink, 1990). The clustering threshold was set to 93% sequence similarity, and only loci present in at least 70% of individuals were included in the assembly.

Data Analysis

To compare levels of genetic diversity in populations of *E. soledium* and *E. shockleyi*, I calculated nucleotide diversity (Nei and Li, 1979) using the R package *PopGenome* (Pfeifer et al., 2014). I also used *PopGenome* to estimate GST (Nei, 1973), a derivative of Wright's FST (Wright, 1965), used

to measure levels of population divergence between populations. I explored the levels of heterozygosity of loci across individuals estimated during the data assembly process in *iPyRAD*. I conducted an unequal variances t-test to evaluate the difference between mean estimated heterozygosity across individuals in *E. soledium* and *E. shockleyi*.

I performed a Mantel Test to search for isolation by distance (IBD) patterns in *E. shockleyi* using the R package *adeigenet* (Jombart, 2015). Genetic and geographic matrices (representing Edwards' and Euclidean geographic distances, respectively) were tested for correlation. 999 replicates were run without the influence of population structure, and the output was compared to the actual correlation between geographic and genetic distances in my data. Because a pattern was detected, genetic and geographic data were plotted to determine the nature of the IBD.

Assembled SNP data was then evaluated using the program *STRUCTURE* (Pritchard, Stephens, and Donnelly, 2000) to explore the number of functional groups, and the extent of admixture between them. Because of its functionality in dealing with admixed individuals, *STRUCTURE* is used for studying genetic relatedness. *STRUCTURE* uses a Bayesian clustering method to statistically assign individuals to source populations based on genetic data. At the same time, the group of allele frequencies for each population is estimated. The model makes several assumptions: within-population Hardy Weinberg equilibrium, within-population linkage disequilibrium, and SNPs are assumed to be unlinked. I ran 20 independent replicates for each possible value for K (groups or clusters) 1 through 20, with

10,000 burn-in steps and 100,000 search steps. Results were assembled and summarized using *CLUMPP* (Jakobssen and Rosenberg, 2006).

RESULTS

237 million raw reads (823,000 loci) were sequenced, 234 million reads (789,000 loci) remained after filtering for quality, and 9.4 million reads (211 loci) remained after filtering to remove loci not present in at least 70% of the individuals. The average read depth was 61.2 reads per SNP, and the average sequencing error rate across samples was 0.0035. There are several possible reasons for the amount of data lost when this last filter was applied. The size selection step prior to sequencing is one possible source of error (Peterson et al., 2012; DaCosta and Sorenson, 2014). PCR amplification, by nature, is biased towards the amplification shorter DNA sequences (Aird et al., 2011). If the size selection failed, many short, random sequences would be retained, and sequenced. In this situation, there is a low probability that these numerous short fragments contain loci represented in the majority of individuals. This problem is exacerbated in large genomes, because the probability of sequencing the same loci across many individuals is further reduced. Additionally, *EcoR1* is a methylation-sensitive restriction enzyme (McClelland, 1981), and patterns of methylation are unknown in most plant taxa. Methylation-sensitive restriction enzymes preferentially exclude undesirable, repetitive regions of the genome (Parchman et al., 2012). It is possible that these species have low amounts of methylation, reducing the shared coverage of loci. Another potentially cause for the low number of loci retained is sequencing depth. I included 288 samples in one sequencing lane,

which likely explains the shallow sequencing depth. Shallow coverage would reduce the likelihood that a locus is sequenced in more than 70% of the individuals. However, 211 variable sites is a significant amount of information. The raw DNA sequence reads and details of all analyses are available at Digital Commons.

Genetic Diversity

Nucleotide diversity (Nei and Li, 1979) for *E. shockleyi* was estimated at 1.82, whereas that in *E. soledium* was found to be 0.21. GST, a statistic used to measure divergence between groups, was estimated to be 0.18. The mean estimated level of heterozygosity across loci for individuals of *E. shockleyi* was 0.016 (sd = 0.0031), while that in *E. soledium* was 0.013 (sd = 0.0033). An unequal variances t-test determined the two means to be significantly different ($p = 1.19e-06$; 95% confidence interval for the true difference between the two means = 0.002-0.004). Figure 3 illustrates the higher levels of heterozygosity in *E. shockleyi* compared to *E. soledium*.

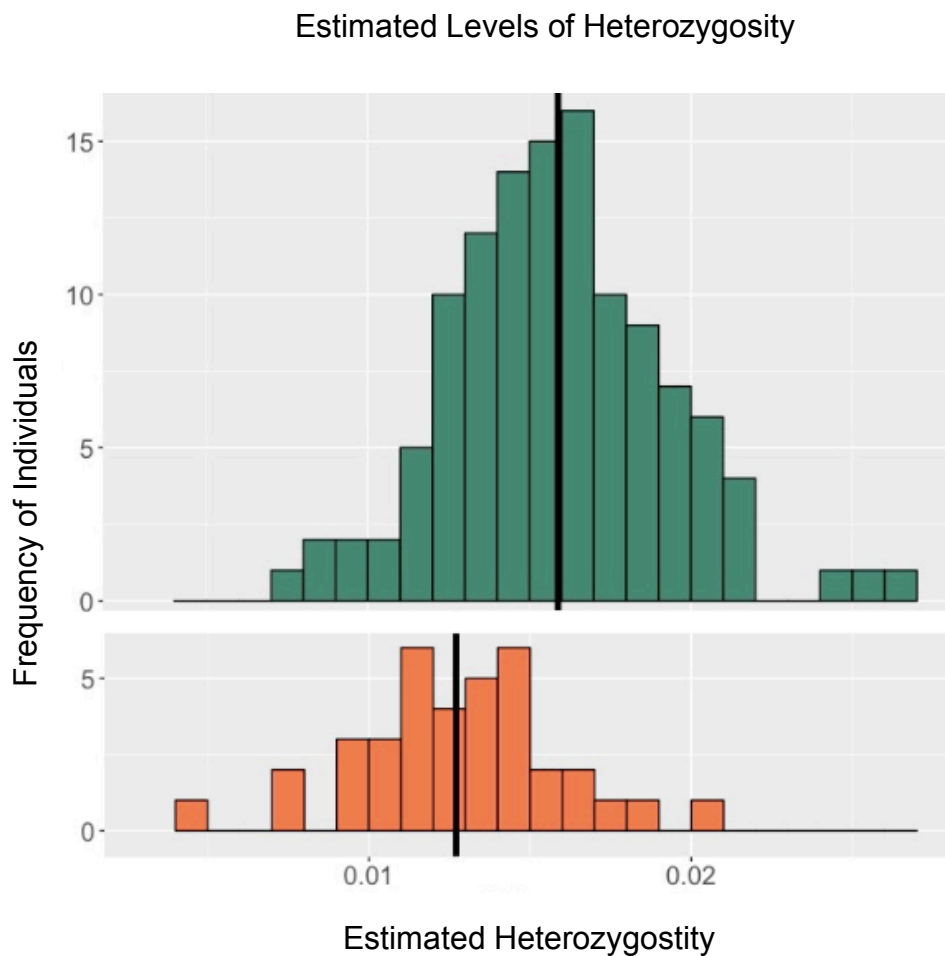


Figure 3. Distribution of the frequency of individuals with increasing levels of mean heterozygosity in *E. shockleyi* (teal) and *E. soledium* (orange). The mean for each distribution is shown with a vertical black line.

A Mantel test revealed a regression coefficient of 0.28. The relationship between genetic and geographic data for *E. shockleyi* fell outside of the range of the simulated values (p value = .001). This indicates the presence of isolation by distance (Fig. 4). As expected, when geographic distance between individuals increases, the probability of reproduction between them decreases.

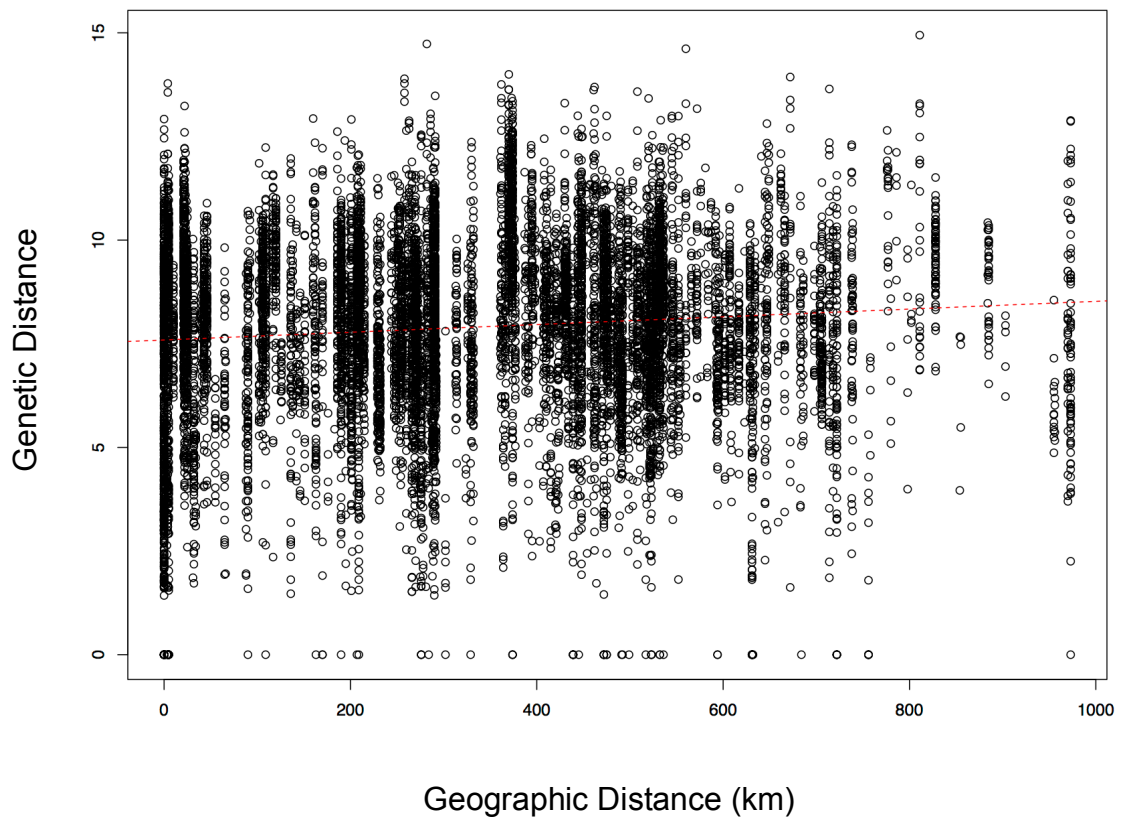
Isolation by Distance in *E. shockleyi*

Figure 4. A Mantel test to investigate the relationship between genetic and geographic distances, to highlight the pattern of isolation by distance in *E. shockleyi*.

Inferring Population Structure

Structure analysis shows moderate levels of admixture between populations of *E. shockleyi* and *E. soredium*. Here I consider individuals with a contribution of more than 10% genomic composition to be admixed. I found that 40.5% of individuals morphologically identifying as *E. soredium* show significant admixture, while 20.7% of *E. shockleyi* appear to be admixed. I found only 59.5% of the individuals of *E. soredium*, and 72.9% of *E. shockleyi*, to contain more than 90% genome composition matching the species to which

they were morphologically identified. In addition, only 4.2% of individuals that were identified morphologically as *E. shockleyi* reported a higher genomic composition of *E. soledium*, while 27.0% of *E. soledium* had discordant results. However, almost half of discordant samples of *E. soledium* came from a single population (population 29), in which 5 out of 6 of the individuals report higher genomic composition of *E. shockleyi*. With this population excluded, only 16.1% of the *E. soledium* individuals have incongruous results.

Figure 5 shows that as the number of source populations, or clusters (K) recognized by *STRUCTURE* is increased, the genomic composition of individuals of *E. soledium* continue to cluster together, while individuals of *E. shockleyi* separate into more and more clusters. This implies that *E. shockleyi* has a complex genetic structure, perhaps due to hybridization with other species throughout its broad geographic range.

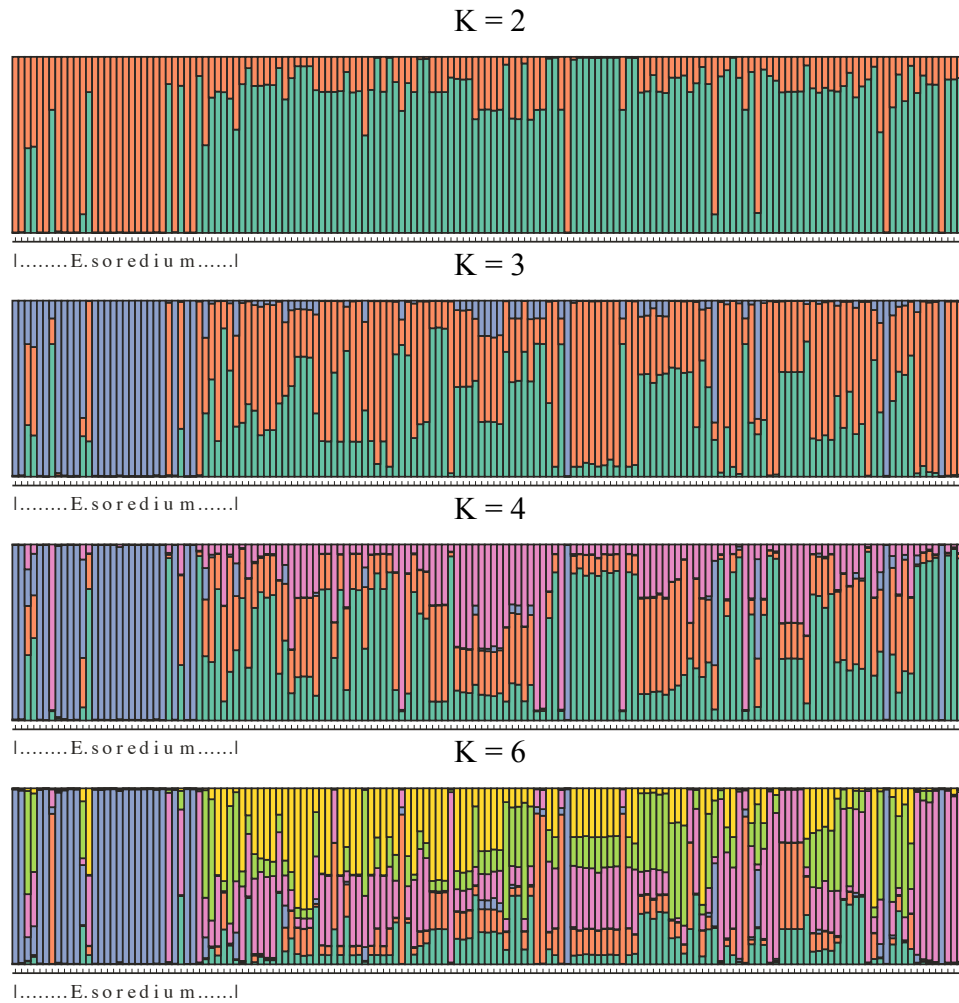


Figure 5. Proportions of admixture based on *STRUCTURE* analysis of SNP data. Samples are arranged in populations, first of *E. soredium*, followed by *E. shockleyi* (left to right). Each bar represents an individual, and the distribution of genetic composition is broken down by color. The number of source populations, or clusters (K) observed by *STRUCTURE* is increased sequentially.

DISCUSSION

The genome of *E. shockleyi* appears to be extremely heterogeneous. Analyses from this study, and others (Smith and Bateman, 2002; Grady, 2012) suggest that *E. shockleyi* is forming hybrids with other species of *Eriogonum* across the western United States. The mosaic composition of *E.*

shockleyi likely represents the influence of other species in the *Eucycla II* subgenus through hybridization (Grady, 2012). This hypothesis is supported by the division of genome composition observed in *E. shockleyi* under an increasing number of assumed source populations, while *E. soledium* continues to cluster into one group. Further insights might be gathered by investigating the relationship between *E. shockleyi* and other members of the *Eucycla II* subgenus (*Eriogonum pelinophilum*, *E. clavellatum*, *E. lonchophyllum*, *E. gracilipes*) with which it could be hybridizing (Grady, 2012).

The broad geographic spread of *E. shockleyi* also maintains diversity in the species through genetic drift. I observed patterns of isolation by distance in *E. shockleyi*, indicating a reduced probability of mating as populations become more distant. This geographic structure is commonly seen in plants because of their limited dispersal ability, and reliance on abiotic factors for reproduction (Levin and Kerster, 1974). Reduced levels of gene flow likely have allowed differences to accumulate between populations, and could partially explain the diversity in genome composition found among populations of *E. shockleyi*. The northernmost populations of *E. shockleyi* appear to have experienced the greatest amount of divergence, and have likely been isolated from the rest of the populations by geographic distance. Rapid diversification within the genus *Eriogonum* across the western United States, combined with isolation by distance, could be responsible for expediting genetic drift in this species.

Not surprisingly, the genomic diversity of *E. shockleyi* is unmatched in *E. soledium*. As an edaphic specialist, with a geographic range restricted to a

few mountain peaks, individuals of *E. soledium* have a lower degree of genetic polymorphism. This contrast corroborates the hypothesis of previous studies that *E. shockleyi* and *E. soledium* are a progenitor-derivative pair, with *E. soledium* being a derivative of *E. shockleyi* formed on the basis of substrate differences (Smith and Bateman, 2002; Grady, 2012). In this situation, *E. soledium* would be expected to possess only a subset of the variation found in its progenitor (Kruckeberg and Rabinowitz, 1985). The higher levels of genetic diversity observed in *E. shockleyi* support this theory. However, the results of this study suggest that there may be an intermediate derivative between *E. shockleyi* and *E. soledium*. Hybridization between *E. soledium* and this intermediate species would explain the moderate amount of admixture observed. The distribution of genomic composition between these two species is consistent with the existence of an intermediate derivative.

Although I did not perform reproduction experiments, I found genetic evidence suggesting of gene flow between these two species. Admixture can result when historically isolated populations are reintroduced and begin to hybridize. Introgression introduces genetic material through the repeated backcrossing of a hybrid to one of the parents. Both of these adhesive processes could be blending the genomes of *E. shockleyi* and *E. soledium*, limiting further divergence. The state of genomic composition in the two species implies the presence of limited (but existent) gene flow between them. This situation is common in plants, because rather than reproductive isolation, speciation is initially driven by a positive feedback loop between diversifying selection and genetic divergence (Rajakaruna, 2004; Rieseberg and Willis, 2007; Räsänen and Hendry, 2008). As a result, interspecific hybrids are often

stable, and reproductive isolation is formed slowly by degrees. As demonstrated by *STRUCTURE* analysis, this intermediate state between continued hybridization, and isolation, is reflected in the genomic composition of *E. soledium* and *E. shockleyi*. Because there is overlap in the geographic ranges of the two species, limitations in gene flow are likely the result of sympatric reproductive barriers between them. As reproductive barriers begin to form, gene flow, in the form of admixture and introgression, tears them down. This tug of war between hybridization and speciation is reflected in the intermediate state of divergence between *E. shockleyi* and *E. soledium*.

Nei's G_{ST} revealed moderate levels of genetic divergence between *E. soledium* and *E. shockleyi*. There exists a degree of genetic distinction between them, but reproductive isolation, and complete genetic divergence, have not been reached. It is important to note that without confidence intervals, it is difficult to make accurate conclusions about the level of admixture. The true level of admixture may be lower than my point estimates suggest. However, it appears that the substrate specificity of *E. soledium* results in just enough separation to maintain partial reproductive barriers between the two species, keeping them from settling into a pattern of consistent hybridization or isolation. However, it is important to note that *E. shockleyi* also grows on (but shows no preference for) Ordovician limestone. In one population in this study, which morphologically identified as *E. soledium* (population 29), five out of the six individuals genetically identify as *E. shockleyi*. This population highlights the possibility that morphological appearances in these two species could be (perhaps partly) a function of substrate. The edaphic qualities of the soil on which a plant grows influence

its availability to water and other resources (Raven, 1964; Rajakaruna, 2004). This can alter the plant's size, shape, coloration, and other morphological features by means of phenotypic plasticity (Gratani, 2014). On an evolutionary level, substrate can affect the morphology of a plant through adaptation (Rajakaruna, 2004). It is possible that when *E. shockleyi* grows on Ordovician limestone, one, or both, of these processes result in an even more similar appearance to *E. soledium*. This could explain the discordant morphological features and genomic composition of the individuals in population 29.

Conservation Recommendation

The results of this study suggest that *Eriogonum soledium* shows enough molecular and morphological distinction to warrant continued treatment as distinct from *E. shockleyi*. On the continuum of population divergence, which ranges from no genetic distinction, to complete genetic divergence, these two populations appear to be near the genetic divergence end of the spectrum. The two species also exhibit different morphological characteristics. *Eriogonum soledium* can be distinguished from *E. shockleyi* by its glabrous (rather than pubescent) flowers and achenes, pink to white flower color, smaller leaves forming tighter whorls, and concentrated involucre (Reveal, 1981, 1985; Grady and Reveal, 2011). In addition, *E. soledium* is only found growing on the outcrops of Ordovician limestone. I chose to explore the relatedness of *E. shockleyi* to my target organism, *E. soledium*, due to their proximity and morphological similarity. However, it is possible that *E. soledium* hybridizes with other species in the area. Further

studies investigating other close relatives in the area, especially in search of an intermediate derivative between *E. shockleyi* and *E. soledium*, would shed more light on their evolutionary relationship.

Germination tests to observe the physical properties of *E. shockleyi* as it grows on Ordovician limestone would provide insights into the affect of phenotypic plasticity on the species. Reintroduction studies and germination tests to determine whether *E. soledium* can be sustained on other substrates would be worth pursuing. However, effort should be made to preserve the living populations of *E. soledium*. Species resilience is generally correlated with genetic diversity. The low levels of diversity, combined with the restricted range and soil specificity of *E. soledium*, suggest that the species is at risk of extinction. Although the area is not heavily used for recreation, limestone mining in the San Francisco Mountains encroaches on the number of surviving populations of the species. If the USFW decides to list *E. soledium* under the ESA, limiting the expansion of Ordovician limestone mining in the area will be considered.

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CHAPTER 3

CONCLUSIONS

Eriogonum soledium is a rare species of buckwheat endemic to the San Francisco Mountain range of west central Utah. Because of its restricted range and small number of existing populations, *E. soledium* is a candidate for protection under the Endangered Species Act. However, its relationship with a widespread close relative, *Eriogonum shockleyi*, questions the necessity of protecting *E. soledium* under the ESA. The geographic ranges of the two species overlap, they have similar morphology, and the genus *Eriogonum* is known for its readiness in forming hybrids. In this study I used genomic data to determine the genetic relatedness, and extent of hybridization, between the two species.

Genomic data revealed higher levels of genetic diversity in populations of *E. shockleyi* compared to *E. soledium*. These results were expected, because more individuals of *E. shockleyi* are included in this study, and the range of *E. shockleyi* is broad. The extensive geographic range of *E. shockleyi* creates the opportunity for hybridization with other species of *Eriogonum*, resulting in the introduction of new alleles. In contrast, *E. soledium* is not known to hybridize with other species. The broad range of *E. shockleyi* also introduces various selective pressures from different environmental factors, while *E. soledium* is only influenced by the selective pressures from its narrow habitat. While *E. shockleyi* is common throughout the west, *E. soledium* has a limited number of individuals. The low number of extant individuals and levels of genetic diversity in *E. soledium* suggest that

species may have undergone recent or frequent bottleneck events. Genetic diversity is important to the survival of a species because it allows populations to adapt to environmental changes.

Because the genus *Eriogonum* is known to form hybrids readily, I was not surprised to find evidence of admixture between *E. soredium* and *E. shockleyi*. Genomic data suggests that there is both admixture between the two species, and reproductive barriers forming between them. When the data is clustered into more than two groups, it becomes evident that the genome of *E. shockleyi* is a heterogeneous mixture, while the genome of *E. soredium* continues to cluster into one constant group. A recent study (Grady, 2012) found evidence of *E. shockleyi* forming hybrids with several other species of *Eriogonum*. The mosaic nature *E. shockleyi*'s genome is likely a reflection of hybridization with related species throughout its range.

There are several probable explanations for the relationship between *E. soredium* and *E. shockleyi*. *Eriogonum soredium* probably first diverged from *E. shockleyi* due to specialization for growth on Ordovician limestone. The edaphic differences likely provided sufficient separation to send the two species into a positive feedback loop between diversifying selection and reproductive isolation. In this situation, when reproductive barriers begin to inhibit gene flow, diversifying selection reinforces reproductive barriers. This cycle can eventually lead to speciation. In addition to adaptation for growth on Ordovician limestone, this substrate may affect the appearance of these mat-forming buckwheats through phenotypic plasticity. I discovered one population of *E. shockleyi*, with the morphological characteristics of *E. soredium*, growing on Ordovician limestone in the San Francisco Mountains. This population

illustrates the possibility that the edaphic qualities of Ordovician limestone can also affect the physical appearance of the plant.

In the genomes of diverging plant populations, there are two extreme possible states. At one extreme there is no genetic distinction between the populations, and alleles are frequently exchanged through gene flow. At the other extreme, genomes are completely distinct. At this extreme, reproductive isolation is complete, and alleles have reached fixation. Most natural populations fall somewhere on a continuum between these two extremes. The genomes of *E. soredium* and *E. shockleyi* are no exception, falling intermediate between the extremes. However, the divergence between these two species is closer to reproductive isolation than frequent hybridization. This genomic situation, combined with the fact that *E. soredium* contains only a fraction of the genetic diversity found in *E. shockleyi*, suggests that *E. soredium* is a derivative of *E. shockleyi*. Furthermore, it is possible that there is an intermediate derivative between *E. shockleyi* and *E. soredium*. In other words, *E. soredium* may be a derivative of one of *E. shockleyi*'s derivatives in the area. The northernmost populations of *E. shockleyi* in this study show the least amount of admixture with *E. soredium*. If *E. soredium* diverged from a derivative of *E. shockleyi*, the northernmost populations have likely been diverging from this intermediate derivative longer than the rest of the populations. This theory is supported by the evidence of greater levels of admixture between populations of *E. soredium*, and the populations of *E. shockleyi* that are located near *E. soredium*'s geographic range.

The results of this study suggest that the continued treatment of *E. soredium* as distinct from *E. shockleyi* may be appropriate. It appears that

incomplete reproductive barriers separate the two species from frequent hybridization. However, studies using low-copy nuclear genes to investigate potential intermediate derivatives between *E. shockleyi* and *E. soredium* would provide more information regarding their evolutionary relationship.

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