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Geographic patterns of genetic distribution within Calochortus Gunnisonii in the central and southern Rocky Mountains

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GEOGRAPHIC PATTERNS OF GENETIC DISTRIBUTION WITHIN *CALOCHORTUS GUNNISONII* IN THE CENTRAL AND SOUTHERN ROCKY MOUNTAINS

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ABSTRACT

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Organismal population ranges and genetic architecture have largely been shaped by climatic events. The Quaternary Period (2.6 million years to present) has been characterized by a series of climatic events manifested as Ice Ages. During glacial periods, plants and animals in temperate and arctic regions were restricted to small patches of suitable habitat less affected by expanding glaciers and extreme cooling. These refugia held importance for the persistence of organisms through glacier interphases. Mountain system vegetation in temperate latitudes was affected by patchy glacier patterns that separated some species into multiple refugia. The isolation of such populations has had a profound effect on genetic architecture across the globe. Glacial induced reproductive isolation causes genetic differences to arise and may result in the genesis of new species. Peripatric speciation is a species concept that seeks to explain these geneses and states that species arise when climatic or tectonic events isolate small populations from an ancestral population that differentiate due to no or limited gene flow. Isolation of populations to distinct geographic areas via peripatry exposes populations to local genetic drift and/or selection pressures and the resulting genetic architecture should reflect in a geographically concordant manner.

Within the southern Rocky Mountains of North America, recent glacial patterns were patchy and plant populations were highly fragmented. *Calochortus gunnisonii* S. Watson (Liliaceae) is a common lily with a large range spanning Arizona, Utah, Colorado, Wyoming, Montana, and southwestern South Dakota. Herbarium records indicated disjunctions stemming from intermontane basins where suitable habitat is either too patchy or absent in the current climate conditions. The highly dissected range contains populations restricted to high elevation, "island-like" mountain ranges in the southern Rocky Mountains. Using microsatellite data, this research investigated the role of glacial oscillatory demographic changes in the central and southern Rocky Mountains in shaping genetic structure of populations across multiple montane disjunctions.

Previous genetic studies of *Calochortus* are limited to AFLP and chloroplast DNA analyses. Neutral, codominant markers, such as microsatellites, are lacking for the genus. Here, 13 novel species-specific microsatellites were designed for analysis of *C. gunnisonii* populations within mountain ranges from Colorado, Wyoming, and South Dakota. All thirteen microsatellite loci were polymorphic for *Calochortus gunnisonii* and used to analyze 616 individuals spanning seven mountain ranges and 25 populations in the southern Rocky Mountain cordillera. Genetic analyses displayed large amounts of diversity and structure for each population and region. Patterns of relatedness between regions indicated recent colonization and diversification. Furthermore, genetic clustering of populations suggested that multiple areas within the region have served as macro and microrefugia for *C. gunnisonii* during Pleistocene glacial events. *Calochortus gunnisonii* appears to have resided in multiple montane refugia in the southern Rocky Mountains during warm interphases of the Earth's glacier cycles. Here, four refugia were proposed

including the Laramie Complex, Sierra Madre/Medicine Bow Complex, Central Colorado Complex, and the Absaroka region. Long-term isolation results in divergence patterns between refugia and complex patterns of admixture during cooling phases are evident from the genetic data. This lily also inhabits elevations beyond the border of boreal forests on the periphery of mountain ranges. It is this pattern that may be of value as an indicator for recolonization routes of the region for higher alpine floras in future genetic studies.

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CHAPTER I

INTRODUCTION TO THE STUDY SYSTEM: *CALOCHORTUS GUNNISONII* IN THE CENTRAL AND SOUTHERN ROCKY MOUNTAINS

Introduction

This research investigates the distribution of variation in microsatellite loci for a charismatic lily species in the central and southern Rocky Mountains. *Calochortus gunnisonii* S. Watson, Liliaceae, inhabits a large range within the central and southern Rocky Mountains. This lily can be found in woodland meadows, springs, montane woodlands, and forest understories upon mountainsides from 1200-3300 meters (3400-11,000 feet). Despite its wide distribution, the heterogeneous nature of the Rocky Mountains creates disjunctions caused by geographic barriers below and above certain altitudes (i.e., desert basins or alpine tundra). This project was designed to genetically investigate the disjunct populations of *C. gunnisonii* within the Rocky Mountains of Colorado, Wyoming, and the Black Hills of South Dakota. Heavy oscillations of cooling and warming during recent ice ages have likely had a profound effect on population genetic structure for many plant species within the Rocky Mountain cordillera. Expansion and contraction of population ranges during these ice ages has led to the current phytogeography of the Rocky Mountains and untangling complex evolutionary histories is important for furthering our understanding of evolution. Chapter I will serve

as an introduction to the climatic fluctuations of the Quaternary and how organismal populations responded, effects on temperate mountain system flora, and the synergistic effects of these phenomena on genetic architecture. A presentation of the genus *Calochortus* Pursh (Liliaceae), its history, and utility to evolutionary studies within the region will be given. An ecological and geographical characterization of the primary study species, *Calochortus gunnisonii*, will be used to propose this species for the phylogeographic assessment of glacial refugia in the Rocky Mountain Cordillera. Finally, a brief description of the investigation's aims and methodologies will be given in order to set the stage for further chapters.

Species Distributions And Genetic Architecture Molded by Global Climate Change During The Quaternary

Glacier Events Over The Last 2.6 Million Years

Studies of the paleoclimate have increased over the past few decades and multiple disciplines have combined to generate valuable data helping to elucidate the dynamic history of the Earth's landscape (Song et al., 2006; Hewitt, 2000). Cyclic climate variations are tightly tied to the Earth's orbit around the sun and the synergistic relationship creates what are known as Milankovitch variations (Ehlers et al., 2011). Milankovitch variations (MV) are typically manifested as 'Ice Ages' in which glacial events extend across large areas of the Earth's surface. The Quaternary Period (~2.6 MYA to present) is composed of the Pleistocene (~2.6 to 0.8 MYA) and Holocene (~0.8 MYA to present) epochs. During the early Pleistocene, MVs were characterized by 41,000-year (41 ka) precessions of cold and warming phases (Ehlers and Gibbard, 2011).

However, a majority of these cycles rarely reached temperatures cold enough to sustain large continental glaciation events (27 of 41 cycles) (Ehlers and Gibbard, 2007). About 1.2 MYA, a transition in the dominant orbital cyclicity swept MVs to 100 ka cycles and were fully established by ~800 ka ago (Ehlers et al., 2011). The extra 59,000 years created longer expanses of cold periods (~80,000 ka) and larger glacier expansion zones were no longer limited to arctic regions as evidenced by mountain glaciation at lower latitudes. Evidence shows that there were at least 5-6 major glacial events limited to the last 900 ka, and ~20 similar events over the span of the Quaternary Period (Gibbard and Cohen, 2008). The cyclic variations that characterized the Quaternary began in the Tertiary period (~65 to 2.4 MYA) when a general cooling occurred and began to increase in amplitude over time (Ehlers et al., 2011).

Climate Effects On Organismal Populations

Extant organismal population ranges have largely been shaped by climatic events especially those in temperate and arctic regions (Hewitt, 2000). During the long glacial periods of the last 2.6 million years, populations of plants and animals were restricted to small patches of suitable habitat less affected by expanding glaciers and extreme cooling. These refugia were typically separated from one another with reproductive isolation having a profound effect on the genetic makeup of organisms that survived the climate oscillations (Hewitt, 2000; Holderegger and Thiel-Egenter, 2009). Refugia have been of particular interest within the field of biogeography of mountain systems since the early 20th century. Mountain systems were typically covered in large ice sheets during cooling cycles and biogeographers have been interested in determining where mountain species

survived during the ice ages. Three general hypotheses exist that probe this question: did organisms persist outside the mountain systems 1) by being displaced to lowland habitat 2) on the periphery of these mountain systems, or 3) at high elevation nunataks (peaks, hills, or ridges protruding from a glacier's surface) within mountain systems? These different types of refugia and their influence on organismal distributions and diversity have been highly debated (reviewed in Holderegger and Thiel-Egenter, 2009). Molecular analyses reawakened interest in the biogeography and phylogeography of mountain ranges. As a result, in-depth hypotheses have been proposed and investigated related to animal and plant distributions in mountain systems over the last fifteen years uncovering striking evolutionary histories of organisms around the globe (reviewed Soltis and Gitzendanner, 1997; Abbott and Bachmann, 2003; Comes and Kadereit, 2003; Schönswetter et al., 2005; Schmitt, 2007).

European Patterns Of Glaciation

Phylogeographic relationships among plants, in respect to climate oscillations, have been well documented over the last half-century in European studies (Bettin et al., 2007; Comes and Kadereit, 1998, 2003; Ehrich et al., 2007; Hewitt, 2004; Stehlik, 2000; Taberlet et al., 1998;). Evidence shows that species found safe harbor in refugia at the periphery of ice sheets or in glacial nunataks in the core of European mountain systems. The European ice sheet spread southward to 52°N, and the permafrost zone reached 47°N (Ehlers and Gibbard, 2007; Hewitt, 2004). This large area exterminated and/or fragmented many organisms' habitats within the region, resulting in drastically reduced distributions. Molecular and fossil records show that species refugia were common in southern Europe, and northward recolonizations during warming periods were rapid

(Hewitt, 2004). Southern peninsular refugia have been found in Iberia, Italy, the Balkans, Greece, and the Caspian/Caucasus regions (Bennett, 1997; Hewitt, 1999). Temperate species in these regions display geographically structured genomes harboring a variety of alleles. The accumulation of these alleles has been attributed to species survival in refugia throughout multiple ice ages (Hewitt, 1999). Specific examples include pond turtle (*Emys orbicularis*; Lenk et al., 1999), hedgehog (*Erinaceus*; Santucci et al., 1998), grasshopper (*Chorthippus*; Hewitt, 1999), bear (*Ursa arcticus*; Hewitt, 1999), and an alpine plant (*Senecio halleri*; Bettin *et al.*, 2007) whose genomes contain distinct genetic signal associated with their respective peninsulas and the repeated use of refugia over multiple glacial cycles (Hewitt, 2004). The genetic consequences of the climatic oscillations may have been significant within the Pleistocene and Quaternary due to repeated allopatry and sympatry events in which populations of organisms were divided in areas where ice expansions and reductions were common. Rapid and heavy divergence may have sped up via selection for specific adaptations within newly colonized, heterogeneous habitats (Hewitt, 1996, 1999).

North American Patterns Of Glaciation

North America was also affected by glacial ice advances but in different ways than Europe. A large ice sheet known as the Laurentide glacier covered much of Canada and parts of the United States, with its deepest southern run extending beyond modern day Chicago. Although this large ice sheet covered some northern portions of North America, the continent generally was characterized by a glacial patchiness at lower latitudes south of the Laurentide ice sheet. In the western United States, the Laurentide

ice sheet touched northern portions of Montana and Washington, but major glaciation originated from high altitude glaciers within the Rocky Mountains and coastal ranges (Hewitt, 2004). It is this patchiness that has resulted in dynamic organismal distributions. In the southeast region, high species richness has been attributed to the presence of refugia south of the continental ice sheet (Avise et al., 1987; Hewitt, 2004). The west, arctic, northwest, and southwest regions display different biotas and phylogeographic patterns due to distinct geographies and climatic changes (Hewitt, 2004). Multiple refugia have been suggested such as the Beringia (Alaska), the central and southern Rocky Mountains, and coastal regions to the southeast and west of the ice sheets (DeChaine and Martin, 2005). Previous molecular and floristic studies have found that the populations inhabiting the Rocky Mountains contain high levels of variation attributed to the Pleistocene climatic conditions (Golden and Bain, 2000; DeChaine and Martin, 2005; Dobeš et al., 2004; Hadley, 1987). Conditions during this epoch promoted persistence and differentiation of the organisms residing in the region.

Study System

The Rocky Mountain System

In western North America, a large mountain range, the Rocky Mountains, extends from New Mexico to northern Canada. This mountain system is the oldest mountain range in western North America and attained its highest relief during the Eocene (45-36 MYA). Current elevations range from about 1000 to 4000 meters (Brunsfeld and Sullivan, 2005). The cordillera can be divided into three physiographic regions: the Northern Rockies from Canada to northern Montana, the central Rockies from Montana to the Wyoming Basin, and the southern Rockies from the Wyoming Basin to New Mexico (DeChaine

and Martin, 2005). Topography and climate are heterogeneous across the Rocky Mountain cordillera and extant population structures may signal episodic population contraction and expansion characteristics for individual species mirroring that of glacial cycles across the Pleistocene and Holocene epochs (DeChaine and Martin, 2005; Funk et al., 2005; Hewitt, 2004). During the last glacial maximum (LGM), the Cordilleran and Laurentide ice sheets covered much of the northern Rockies into northern Montana. The central Rockies were covered in a roughly 1000m thick layer of ice (Pierce, 1979) and the southern Rockies were characterized by less continuous, more localized montane glaciers (Elias, 1996; Leonard, 2007). Mountain ranges in the south-central and northern portion of the southern Rockies include the Absaroka Range, Big Horn Mountains, Black Hills, Laramie Mountain Range, Sierra Madre Mountains, Medicine Bow Mountains, Elk Mountains, Gore Range, Flat Tops, Rabbit Ears Range, Mosquito Range, Sawatch Range, Sangre de Cristo Range, San Juan Mountains, and the Front Range. The Black Hills represent the eastern most portion of the Rocky Mountain uplift (Zaprowski et al., 2001). A large intermontane basin, known as the Wyoming Basin, stretches from east to west across much of Wyoming and separates the central and southern Rocky Mountains. This basin also cuts into small areas between mountain ranges across the state and its harsh, arid climate is not currently suitable for high elevation plants. Ranges at the northern portion of the southern Rockies, and southern Wyoming Basin, such as the Laramie, Medicine Bow, and Sierra Madre Ranges represent the northern edge of the southern Rocky Mountain system. The Black Hills, Big Horns, and Absaroka Ranges represent the southern edge of the central Rockies (Figure 1).

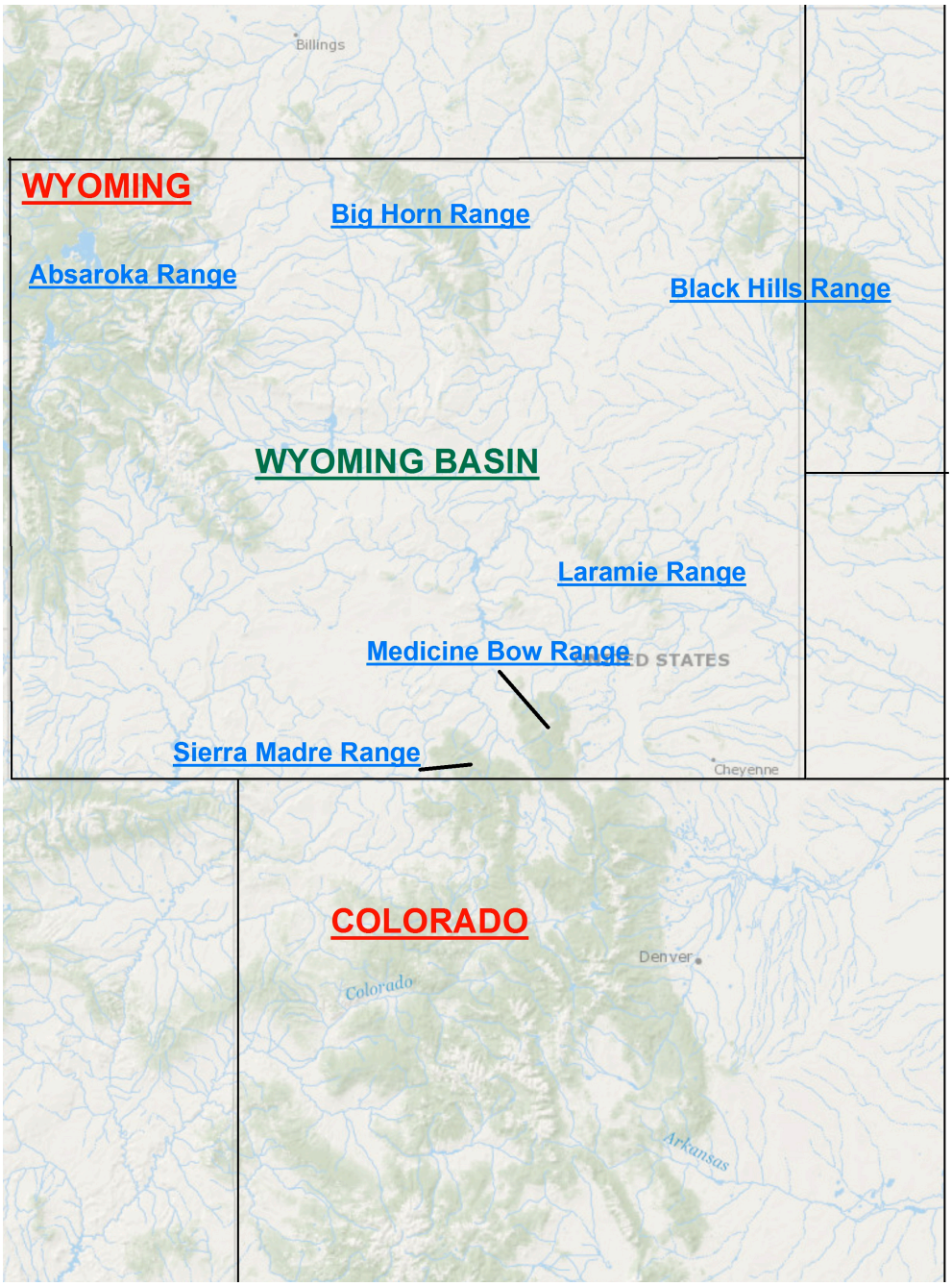


Figure 1: Map displaying the Wyoming Basin and the bordering mountain ranges of concern in this study (Base map from ArcMap 10.1).

Genetic consequences of prehistoric climate patterns have led to many theories of how genetic architecture and species richness would present itself across the extent of the Rocky Mountain cordillera (Hadley, 1987; Hewitt, 2004; Nielson et al., 2001; Rull, 2009; Song et al., 2006). In general, temperate species across the globe display less genetic variation in the higher, colder latitudes to which they expanded after the last ice age. However, this can be dependent on the particular niche, geography, and biology of individual species (Hewitt, 2004). Regions may also be colonized by species whose genomes come from a diverse set of refugia, also known as micro or macrorefugia (Hewitt, 2004; Rull, 2009). Disjunct, divergent populations can provide evidence of range changes in earlier interglacial periods (Hewitt, 2004). The extent of this genetic divergence is unique from species to species and is a measure of the time since their initial separation. In areas where lineages are able to persist through multiple climatic oscillations, genetic differences can accumulate, leading to potential speciation events (Hewitt, 2004). This type of montane divergence phenomena can be observed in North America with tailed frogs (*Ascaphus truei*: Nielson et al., 2001) and in southern European mountain systems with Iberian lizards and beetles (Hewitt, 2000).

Expansions and contractions of glaciers in mountain systems can have profound effects on local plant distributions (Holderegger and Thiel-Egenter, 2009). As cooling persisted and mountain peaks became glaciated, populations would have been exterminated or forced to retreat downward into lower altitudinal and latitudinal refugia. However, in warming periods, lowland, peripheral, or nunatak micro- or macrorefugia were sources of plant colonizations across broad regions (Hewitt, 2004; Holderegger and Thiel-Egenter, 2009; Rull, 2009).

In our current warming period, the Wyoming Basin represents a wide desert characterized by low levels of precipitation and extreme temperature ranges (Hadley, 1987). The 'island-like' mountain ranges found within this basin, such as the Big Horn Mountains and the Black Hills, may represent refugia for some high-elevation plant and animal species (Thomasson et al., 2006). Furthermore, during glacial periods, Nearctic deserts such as these may have contained refugia for retreating organisms (Wilson and Pitts, 2012). Alpine floras of the central and southern Rockies share similar species that are tightly correlated to the size of alpine habitat on a given peak and distance to nearest neighbor (Hadley, 1987; Thomasson et al., 2006). Current restrictions of alpine floras to patchy peaks throughout the region are a characteristic of warm, interglacial periods. In cooling periods, connection of alpine habitat was not uncommon, especially in the southern Rockies (Hadley, 1987). Furthermore, middens have provided fossil evidence of *Pinus ponderosa* within the basins of Wyoming (Norris, 2006; Wells, 1970). These fossils suggest that open forest woodland once stretched across portions of central Wyoming and probably connected the disjunct mountain ranges to one another at various times. Corridors have been proposed that would allow for the diffusion of non-long distance dispersal plants to reach northern latitudes (postglacial suture zones) (Weber, 1965; Billings, 1978). Further support for these corridors can be found in the mountain vegetation, which is usually most closely related to adjacent lowland vegetation (Hadley, 1987). The Rocky Mountain and Great Basin floras are strongly similar and diffusion, not long distance dispersal, has been proposed as the dominant mechanism of range expansions within the Great Basin region (Billings, 1978).

Molecular and paleoclimatic studies suggest that the central and southern Rocky Mountains harbored important glacial refugia for the high-latitude and high-altitude flora in North America (Song et al., 2006; Brunsfeld and Sullivan, 2006; DeChaine and Martin, 2005; Good and Sullivan, 2001; Li and Adams, 1989; Mitton et al., 2000). Organelle, isozyme, and microsatellite techniques used in these studies suggest multiple Rocky Mountain refugia spanned the Rocky Mountain cordillera. It is clear that climatic events of the last several million years have shaped the phytogeography of the Rocky Mountains, and the dynamics of species responses are highly variable within the oscillations (Song et al., 2006; DeChaine and Martin, 2005; Elias, 1996; Hadley, 1987; Hewitt, 1996, 1999, 2004; Pierce, 1979). Each organism inhabiting this region has most likely undergone a turbulent genetic history and molecular analyses may assist in elucidating how different organisms responded to extreme conditions of ice age fluctuations.

The Genus *Calochortus*

Calochortus Pursh (Liliaceae) is a large genus of bulbous geophytes (ca. 70 spp.) originating in California ~7 million years ago (Patterson and Givnish, 2002). Its range includes a center of diversity in California that spreads north to British Columbia, east to the Dakotas, and south to Guatemala (Ownbey 1940; Patterson and Givnish, 2003; Henss et al., 2013). Four distinct floral syndromes are present within the genus (mariposas, star tulips, fairy lanterns, cat's ears) and diverse pollinators are known to visit many species (Dilley et al., 2000; Jokerst, 1981). The genus also occurs in a wide range of habitats including grasslands, deserts, vernal pools, woodland meadows, springs, montane woodlands, and forest understories with most taxa occupying narrow geographic ranges

(Ownbey, 1940; Patterson and Givnish 2004; Fiedler and Zebell, 2012; Henss et al., 2013).

Patterson and Givnish (2003) used chloroplast DNA (cpDNA) sequence data to describe seven major clades from different geographic regions within *Calochortus*. The cpDNA analysis revealed a major hub of diversity for the genus exists in California (identified clades include: Bay Area (10spp.), San Diego (5 spp.), Southwestern California (5 spp.), and the Coast Ranges/Sierra Nevada (6 spp.)) (Patterson and Givnish, 2003). Other identified clades include the Great Basin-Rocky Mountain (11 spp.), Central Mexico (13 spp.), and Pacific Northwest clades (17 spp.). Further analyses suggest that independent evolution of habitat preference, floral syndrome, and serpentine tolerance has occurred several times over small spatial scales possibly due to localized adaptations. Furthermore, *Calochortus'* reproductive biology suggests that seed movement occurs over small spatial scales due to large seeds lacking obvious morphological adaptations for long-distance dispersal (with the exception of winged seeds in *C. macrocarpus*) (Henss et al., 2013). Seeds of *C. westonii* spill within 15 centimeters of the parental plant and create clusters of 20-30 seedlings the following spring (Knapp, 1995). Bullock (1976) showed that seeds of *C. catalinae* and *C. clavatus* move <2 meters after heavy rainfall on an experimental slope. Without external forces carrying seeds long distances, *Calochortus* displays short distance, creeping expansions at the leading edge of populations. This reproductive biology suggests a few scenarios for the current diversity levels across the genus: relatively ancient founder event(s) with selection or drift acting afterwards, pollinator-mediated gene flow, geological phenomena creating population disjunctions (i.e. uplift of the California coast ranges 3-5 million years ago), and/or

climate events isolating populations (i.e. glaciation). Uplifts of the ranges along the California Coast have been proposed as major facilitators of divergence events within *Calochortus*, which prevented successful sexual reproduction among divergent lineages. Over time, limited dispersal led to narrow endemism, parallel radiations in habitat colonization, serpentine tolerance, and distinctive floral morphologies (Patterson and Givnish, 2003). Furthermore, chromosome shifts are common in this genus and combined, these phenomena seem to have led to reproductive isolation and overlapping ranges for many species within the genus (Patterson and Givnish, 2003).

Scenarios where limited gene flow via seed dispersal over small spatial scales is present results in narrow endemism, geographic coherence of individual clades, and parallel adaptive radiations (Patterson and Givnish, 2003; Henss et al., 2013). Additionally, this results in spatial genetic structure over short distances within species and can ultimately drive speciation and endemism over limited geographic scales (most notable in the California clades) (Patterson and Givnish, 2004; Henns, 2013). However, the Great Basin/Rocky Mountain clades contain some generalist species that inhabit a wide range of habitats including deserts and dry grasslands (*C. ambiguus*, *C. kennedyi*, *C. macrocarpus*, and *C. nuttallii*) and have large ranges in comparison to others in the genus (Patterson and Givnish, 2003; Fiedler and Zebell, 2012). These dispersal and divergence events came after initial colonization from the inferred ancestral distributions originating in the Coast Range group of the California Floristic Province (Patterson and Givnish, 2003).

Species Of Interest

Calochortus gunnisonii S. Watson (Liliaceae) has a large distribution encompassing northeastern Arizona, northern New Mexico, much of Colorado, eastern Utah, large portions of Wyoming, southern Montana, and western South Dakota (Fiedler and Zebell, 2012). This species achieves some of the highest elevations for the genus in the southern portion of the Rocky Mountains from 1200-3300 meters (3400-11,000 feet). *Calochortus gunnisonii* contains 1-3 open, upward-facing flowers on a single flowering stock (Ownbey, 1940). These mariposa flowers are white to purple with a distinctive, transverse purple band distal to a pubescent gland at the base of each petal (Fiedler and Zebell, 2012). One ribbon-like basal leaf is present that originates from a perennial bulb and typically withers at sexual maturation. *Calochortus gunnisonii* possesses the ancestral chromosome number of 2n=18. Patterson and Givnish (2003) found that *C. ambiguus* (2n=18) is the closest sister species to *C. gunnisonii*, uniting them as the only species with this chromosome number in the Great Basin/Rocky Mountain clade.

Calochortus gunnisonii is believed to have colonized the Rocky Mountain range within the last 7 million years and has since expanded to one of the largest distributions within the genus (Patterson and Givnish, 2002; Fiedler and Zebell, 2012). Interestingly, *C. gunnisonii* reaches higher altitudes than a majority of the species and is the highest within the Great Basin clade (Fiedler and Zebell, 2012). Glacial oscillations of the Quaternary may have had profound effects on the genetic structure of this plant, as populations could have been pushed from montane valley to montane valley, across intermontane deserts and basins via available refugia. The central and southern Rocky Mountain system showed patchy ice distribution during recent major glacier events and

the expansion of mountain vegetation downward during cooling periods might have allowed *C. gunnisonii* to disperse down into the surrounding lowlands of the Wyoming Basin. Current herbaria maps favor this scenario and are supported by disjunct populations that exist on mountain ranges at the northern portion of this species' range embedded within and along the fringes of the Wyoming Basin (Figure 2). These include the Medicine Bow Range, Laramie Mountain Range, Black Hills, Big Horn Mountains, and the Absaroka Range (Figure 2). A closer look at this region yields population distributions that display "island-like" patterns in which intermontane basins create distances between populations and may serve as reproductive barriers to gene flow.

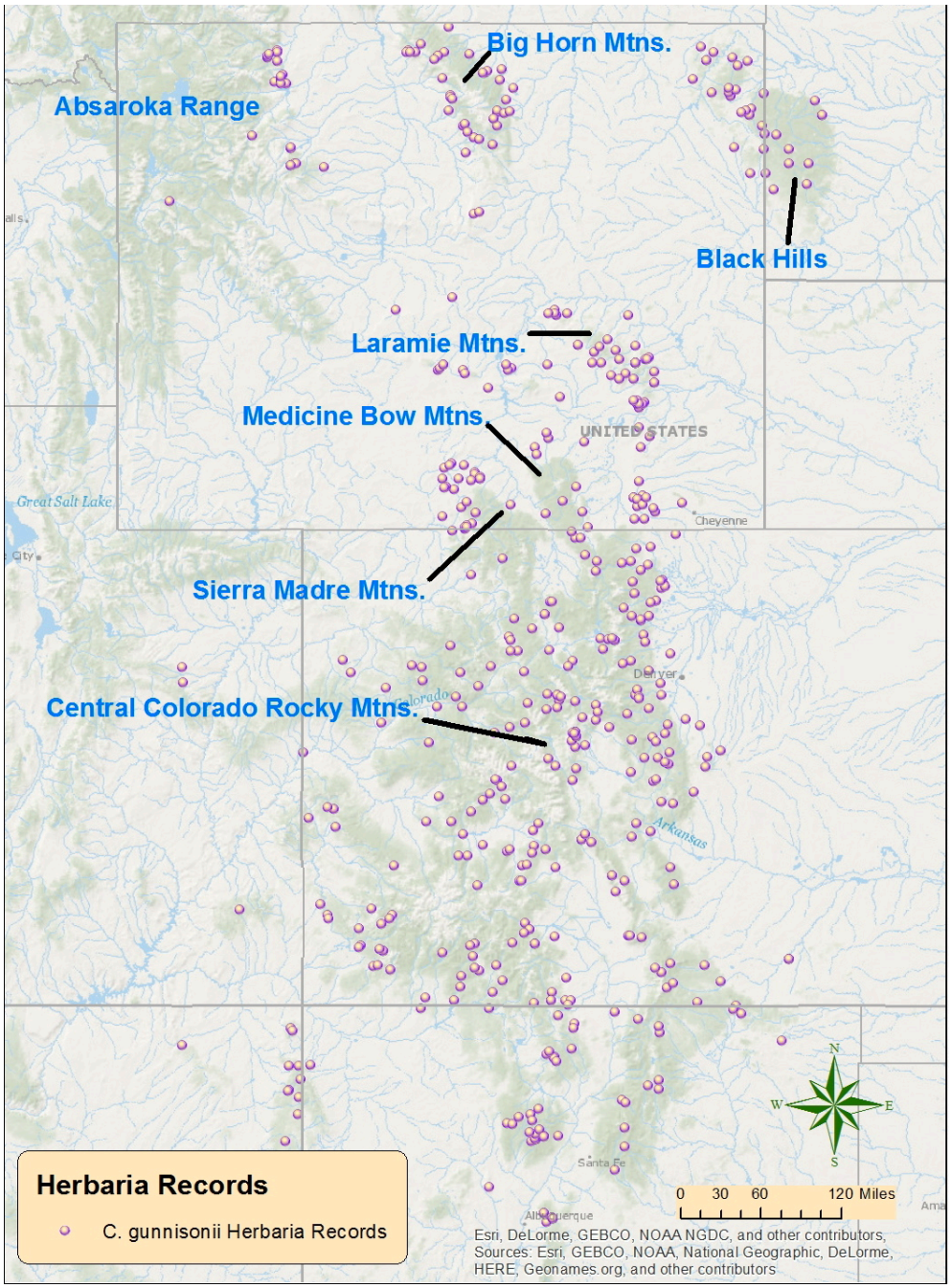


Figure 2: Map displaying the current herbaria records of *Calochortus gunnisonii*. All data points downloaded from swbiodiversity.net and uploaded to ArcGIS10.1 (Base map from ArcGIS 10.1).

Patterns Related To Species Concepts

A primary goal of evolutionary biology is to comprehend the mechanisms that have created species diversity. Peripatric speciation theory states that species arise when small populations become isolated from an ancestral population and differentiate due to no or limited gene flow. Current research describes this model of diverging populations as occurring in one of three ways: 1) colonization takes place as a founder event of a distant habitat, such as a lake or island 2) a geographic barrier may cause a small peripheral population to become isolated from that of the ancestral population 3) a non-peripheral population within a species' range may be separated for a time period long enough for reproductive isolation to occur (Coyne & Orr, 2004). Glacial oscillations can play a role in, or be the cause of, each of these scenarios. For example, colonization of refugia (distant habitat) during glacial expansion, termination of connections between populations by cooling climates or direct glacier intrusion, and the subsequent separation of these populations for long periods of time can create opportunities for reproductive isolation.

Peripatric speciation presents unique challenges for detection on continental landscapes. Island archipelagos are typically chosen due to the ease of tracking founder events. Oceans typically represent stout geographic barriers between islands and are effective at preventing inter-island gene flow. However, islands only constitute a small sampling of available habitat and continental peripatric speciation is difficult to detect. The geography of continental landscapes is dynamic and organismal populations are consistently fragmented through natural means, such as glaciations. The geography of

North America, in particular the United States, presents a suitable candidate for many continental studies of peripatric speciation due to numerous geographical opportunities where peripatry may occur. To the east, the Appalachian Mountain chain divides the eastern seaboard from the Great Lakes and Mississippi River Basin. In the west are the Great Plains and a geographic anomaly known as the Black Hills, which contains the highest peak east of the core Rocky Mountains. The large geographic area of the Great Plains ends abruptly at the base of the Rockies. The Intermountain West consists of arid desert landscapes that lead to the Cascade and Sierra Nevada Mountain ranges. However, detailed scenarios of peripatric speciation can be hard to detect in glacier-affected areas due to the expansion and contraction of population edges. In fact, frequent glacier expansions and contractions in mountain areas have made it difficult to accurately date major events because evidence of these events can be destroyed when glaciers expand and contract (Ehlers et al., 2011). If sufficient time has not passed between isolated populations, 'genetic rescue' occurrences may blur the lines between diverging populations. The reconnection of larger gene pools to disjunct populations may eliminate unique, detectable genetic differences between populations (i.e., private alleles) and thus can make declaring peripatric speciation as a mechanism of divergence difficult in some cases (Coyne and Orr, 2004).

Peripatric speciation predicts that climatic and/or tectonic events fragment populations and reproductive traits of isolates (Seddon and Tobias, 2007). Evidence shows that historical glaciation events shape modern vegetation patterns in many areas of North America (Pielou, 1991). Mountain ranges on the northeastern edges of *C. gunnisonii's* range display similar patterns. These phenomena are thought to have

displaced many species in the region. At lower elevations between the Rocky Mountains and the disjunct mountain ranges of Wyoming and South Dakota, higher elevation species tend to be absent. *Calochortus gunnisonii* populations present a suitable opportunity to genetically investigate peripatric divergence. A prerequisite to speciation via peripatry is time. Populations of species in allopatry require time and evolutionary forces such as drift, selection, and mutation to become reproductively isolated and generate distinctively new species (Coyne and Orr, 2004). Prehistoric glacial events in the Rocky Mountain region may have played a key role in current peripatric distribution patterns for *C. gunnisonii* in one of two ways: (1) historical, montane glaciation and expansion of alpine zones into surrounding lowland areas (i.e., Pawnee National Grasslands, Wyoming's intermontane basins) within the region pushed *C. gunnisonii* down to lower elevations, and as warmer temperatures prevailed, retreating glaciers and newly available alpine habitats were colonized by *C. gunnisonii*, causing the "island-like" populations to become isolated; or (2) glaciers in the central Rockies may have exterminated *C. gunnisonii* within the Wyoming Basin as inhospitable habitat moved across the Thunder Basin and Oglala National Grasslands, successfully destroying populations connecting the smaller mountain ranges to the Rocky Mountains (Wells, 1970). This latter scenario is not well supported in the literature and would require a significantly large population of *C. gunnisonii* to inhabit regions where it does not currently exist. Furthermore, the absence of large basin populations in the current warming phase of the climate suggests low-elevation genotypes do not persist in the open desert, except in patches of moist habitat containing aspen, pine, and sage species (pers. obs 2013/2014 and herbarium specimens).

The Rocky Mountains and the Black Hills share many plant distribution models similar to that of *C. gunnisonnii* (Thomasson et al., 2006). If sufficient evidence exists of clear differences between the disjunct isolates, then other species in this region may also be under the influence of peripatric speciation and should be managed as unique. This type of evidence could spearhead further research of the special disjunction shared by the central and southern Rocky Mountains.

Study Aims And Methods

This thesis presents an investigation into the evolutionary history of *Calochortus gunnisonii* in the central and southern Rocky Mountain region. The primary research aim of this study is to infer the role of glacial oscillatory demographic changes in the central and southern Rocky Mountains in shaping genetic structure of *C. gunnisonii* populations across multiple montane disjunctions. This will involve analyzing the level of genetic divergence within and among populations and mountain ranges, the degree of gene flow related to geographic structure, and the correlation of peripatric characteristics to this species' history. As in previous molecular analyses, a set of molecular markers will be used to elucidate phylogeography and population history. In Chapter II, a presentation of microsatellite marker design using next generation sequencing techniques will be given. Chapter III of this thesis is a report on recent evolutionary processes affecting genetic variation in the study system using nuclear microsatellite markers. Microsatellites are neutral, biparentally inherited markers that allow for recombination, thus integrating several genealogical processes (Heuertz et al., 2004). Genetic variation at neutral loci across the whole nuclear genome can provide null distributions for recent population processes such as isolation by distance (IBD), gene flow, genetic bottlenecks, and inter

and intra-population genetic barriers. Chapter IV represents a synthesis of the thesis research, placing the results and interpretation into context of previous findings on the flora of the Rocky Mountain cordillera and how this study system plays a large role in elucidating plant evolutionary history in other temperate regions.

CHAPTER II

CHARACTERIZATION OF MICROSATELLITE MARKERS FOR GUNNISON'S SEGO LILY, *CALOCHORTUS GUNNISONII* (LILIACEAE), FROM ILLUMINA MiSEQ SEQUENCING

Introduction

Calochortus Pursh (Liliaceae) is a large genus of bulbous geophytes (ca. 70 spp.) originating in California ~7 million years ago (Patterson and Givnish, 2003). Its range includes a center of diversity in California that spreads north to British Columbia, east to the Dakotas, and south to Guatemala (Ownbey 1940; Patterson and Givnish, 2003; Henss et al., 2013). The genus also occurs in a wide range of habitats including grasslands, deserts, vernal pools, woodland meadows, springs, montane woodlands, and forest understories with most taxa occupying narrow geographic ranges (Ownbey, 1940; Patterson and Givnish 2003; Fiedler and Zebell, 2012; Henss et al., 2013).

Gunnison's Sego Lily, *Calochortus gunnisonii* S. Watson (Liliaceae), is a North American endemic populating portions of the central and southern Rocky Mountains. *Calochortus gunnisonii* has a broad distribution encompassing northeastern Arizona, northern New Mexico, much of Colorado, eastern Utah, large portions of Wyoming, southern Montana, and western South Dakota at elevations of 1200-3300 meters (Fiedler and Zebell, 2012). This species achieves some of the highest elevations for the genus in

the southern portion of the Rocky Mountains. Disjunctions in the northern portions of *C. gunnisonii's* range exist across the Big Horn Mountains, Black Hills, Absaroka Mountains, Sierra Madre, Medicine Bow, and Laramie Range. A population genetic study of *C. gunnisonii* across multiple, disjunct populations in the central and southern Rocky Mountains is currently being conducted. However, previous genetic studies of members within the genus are limited to amplified fragment length polymorphisms (Henss et al., 2013) and chloroplast sequence comparisons (Patterson and Givnish, 2003). Here, we report the characterization of 13 microsatellite loci that will be used to investigate the role of glacial oscillatory demographic changes in shaping genetic structure of *C. gunnisonii* across multiple montane disjunctions of the central and southern Rocky Mountains.

Methods And Results

Next generation sequencing was used to acquire a large quantity of genomic sequence data in search of microsatellite repeats. Genomic DNA (gDNA) was extracted from leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) protocol (Friar, 2005). Two individual DNA samples collected from two separate geographic populations (Black Hills: 43.724753°N, -103.854112°W; La Prele Reservoir: 42.706796°N, -105.578531°W) were pooled and sent to the Center for Genome Research and Biocomputing at Oregon State University. A total of ~400 ng of gDNA was used for library preparation and Illumina MiSeq sequencing (Illumina, San Diego, California, USA). A single, 300bp paired-end MiSeq sequencing run resulted in 18,332,564 reads with an average length of 301 bases. Raw sequence reads were filtered, reformatted, and trimmed using the default commands of the Trimmomatic v.0.32 program (Bolger et al.,

2014). Trimmomatic yielded 12,080,556 high quality fragments (6,040,278 forward and reverse). Fragments were *de novo* assembled using Trinity (Grabherr et al., 2011) producing 486,538 contigs, with an average size of 516 bp (N50=583). MSATCOMMANDER v.1.0.8 (Faircloth, 2008) found a total of 4,118 perfect microsatellite repeats from the assembled contigs: 85 hexanucleotide with at least four repeat units, 585 pentanucleotide with at least four repeat units, 481 tetranucleotide with at least five repeat units, and 2,967 trinucleotide with at least five repeat units. Dinucleotide repeats were excluded to avoid stutter and subsequent scoring problems in later analyses. The inset version of Primer3 (Rozen and Skaletsky, 2000) in MSATCOMMANDER designed 1,153 primer pairs using default parameters (except that GC clamp = yes and repeat motif was ≥4 for penta and hexanucleotide repeats). The PCR product size was set to 120-400 bp. One primer of each pair was designed with a common tag at the 5' end following the procedure of Boutin-Ganache et al. (2001). Two common tags M13R (GGAAACAGCTATGACCAT) and CAGT (CAGTCGGGCGTCATCA), and one custom tag T7 (GCTAGTTATTGCTCAGCGG) were used. Fifty loci with optimum primer conditions were screened for amplification success and within population variability. Microsatellite loci were amplified in 12 µL reaction volumes that included 2.5 mM of tagged primer, 25 mM of non-tagged primer, 25 mM of fluorescently labeled tag (6-FAM, PET, or VIC; Applied Biosystems, Carlsbad, California), 5X Colorless GoTaq Flexi Buffer (Promega, Madison, Wisconsin), 2.5 mM each dNTP, 0.02 U GoTaq Flexi DNA Polymerase (Promega), 0.1 µL 100X Bovine Serum Albumin, 25 mM $MgCl_2$ or 100 mM $MgSO_4$, ~20 to 50 ng of gDNA, and brought to volume with purified water. Thermal cycler conditions were 94°C for 3 min; 35 cycles

of 94° C for 30 s, 56-63°C for 30 s, and 72°C for 30 s; followed by an extension for 10 min at 72°C; and a final 30 min extension at 72°C. PCR products were mixed with 1X SYBR Green (LONZA, Rockland, Maine) and visualized on a 1% agarose gel. Successful PCR products were diluted with water and mixed with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems) before electrophoresis on an Applied Biosystems 3730 Genetic Analyzer at Arizona State University. Fragments were sized using the microsatellite plug-in of Geneious v.8.0.4 (Biomatters, New Zealand).

Of the 50 initial screenings, 22 failed to yield reliable PCR product via gel electrophoresis. Twenty-eight loci yielded consistent amplified products. Preliminary sizing of the products revealed 13 primer pairs that consistently yielded amplicons within the target size range (Table 1). Specific annealing temperatures are reported in Table 1. Three sample populations of *C. gunnisonii* were selected to evaluate variability in the isolated loci. One population represents central Colorado (Shavano Campground; n = 34), the second represents a low-elevation population in northern Colorado (Dixon Reservoir; n = 31), and the third is a population from south-central Wyoming near the northern edge of the southern Rockies (Sand Lake; n = 32).

All 13 microsatellite loci were variable and polymorphic among sampled populations. The mean number of alleles per locus ranged from 3.67 to 6.33, with an average of 4.97 (Table 2). The observed and expected heterozygosity ranged from 0.258 to 0.750 and 0.346 to 0.731, respectively. Significant deviations from HWE were observed for individual loci (Table 2), but no consistent pattern across multiple populations was observed, suggesting the deviations are due to population processes.

Observed and Expected heterozygosities are high when examined across all populations, indicating the value of these markers for future studies.

Conclusions

I identified thirteen *C. gunnisonii* microsatellite loci that are variable and informative. These markers will be used to investigate the population genetic structure and levels of genetic variability of *C. gunnisonii* in the central and southern Rocky Mountains. Intra and intermontane patterns of gene flow and divergence will be inferred within *C. gunnisonii*.

Table 1. Microsatellite PCR conditions for *C. gunnisonii*.

Marker	Anneal Temp (°C)	Magnesium (µL)	Product Size (bp)
CAGU_14	62.4	2.0 Cl_2	255
CAGU_15	62.4	2.0 Cl_2	390
CAGU_22	62.4	1.0 Cl_2	245
CAGU_31	62.4	2.0 Cl_2	320
CAGU_35	62.4	2.0 Cl_2	271
CAGU_36	62.4	2.0 Cl_2	386
CAGU_39	62.4	2.0 Cl_2	347
CAGU_42	66.9	3.0 Cl_2	395
CAGU_45	62.4	3.0 Cl_2	198
CAGU_46	59.8	0.5 SO_4	174
CAGU_47	61.1	1.5 Cl_2	364
CAGU_48	61.1	1.5 SO_4	424
CAGU_50	56.9	3.0 Cl_2	384

*$MgCl_2$ volume stock concentration of 25mM; $MgSO_4$ stock of 100mM.

Table 2. Primer sequences and diversity statistics for 13 microsatellite loci isolated from *Calochortus gunnisonii* (CAGU)

Locus	Primer Sequence (5'-3')	5' Tag	Label Dye	Repeat Motif	Allele Size Range	Pop.	N_A	H_O	H_E	HWE *P value*
CAGU_14	F-TTGTCAAGTGGGCAAGTGTC		FAM	$(ACACC)_4$	246-266	DIX	4.00	0.469	0.469	0.981
	R-ATACAACGCACCGCATACC	M13				SL	3.00	0.233	0.376	0.001**
						SVC	4.00	0.559	0.493	0.968
						Mean	3.67	0.420	0.446	
CAGU_15	F-ATCCTCACTGCCCACTCAC	M13	VIC	$(AGAGG)_4$	385-415	DIX	8.00	0.563	0.607	0.908
	R-GTGCAGCAGATCCACATTCC					SL	4.00	0.419	0.348	0.902
						SVC	3.00	0.571	0.519	0.402
						Mean	5.00	0.518	0.491	
CAGU_22	F-CACATGGTGTTGATGCAGGG		PET	$(ATCC)_5$	237-257	DIX	5.00	0.125	0.252	0.008**
	R-TTGTATCGTGCAACAGTCCC	CAGT				SL	5.00	0.419	0.348	0.902
						SVC	5.00	0.441	0.440	0.457
						Mean	5.00	0.328	0.346	
CAGU_31	F-CCACCCAAGAAGAGCTAAAGG		PET	$(ACATC)_4$	310-340	DIX	6.00	0.531	0.582	0.003**
	R-TTCACCGTCTCCAACAC	CAGT				SL	5.00	0.645	0.674	0.835
						SVC	5.00	0.457	0.602	0.578
						Mean	5.33	0.548	0.619	
CAGU_35	F-TAATACCCGTGAACTCCGGC		VIC	$(ATGC)_5$	263-283	DIX	6.00	0.419	0.601	0.085
	R-TGCAACCGAGTAGTGGACC	M13				SL	5.00	0.241	0.405	0.000***
						SVC	6.00	0.393	0.695	0.000***
						Mean	5.67	0.351	0.567	
CAGU_36	F-CCCTCGATCTCAGCCGATTG		FAM	$(ATCG)_5$	378-398	DIX	5.00	0.387	0.455	0.000***
	R-TAAGTTAGGTACGGAGCAGGC	M13				SL	3.00	0.118	0.213	0.076
						SVC	4.00	0.294	0.407	0.038*
						Mean	4.00	0.266	0.358	
CAGU_39	F-TTACACCAAGCTCCGCAG	CAGT	FAM	$(ACTG)_6$	335-374	DIX	6.00	0.452	0.655	0.473
	R-GTCTTGCTCATGTTGCTCCC					SL	5.00	0.806	0.691	0.943
						SVC	6.00	0.600	0.666	0.000***
						Mean	5.67	0.619	0.671	

Table 2. Continued

CAGU_42	FTCGTGTTCAAGTGCTACCATCG	M13	PET	$(AAGAC)_5$	380-415	DIX	7.00	0.250	0.750	0.000***
	R-CTTGCTGGAATCACATCACCC					SL	4.00	0.448	0.542	0.774
						SVC	5.00	0.077	0.643	0.000***
						Mean	5.33	0.258	0.645	
CAGU_45	F- TCTAGTAGAAACCAGGGTGCC	M13	FAM	$(ACCG)_5$	190-214	DIX	7.00	0.344	0.616	0.000***
	R-ACCCTAGCTCTTGTTCGGTG					SL	4.00	0.548	0.575	0.846
						SVC	5.00	0.500	0.599	0.050
						Mean	5.33	0.464	0.597	
CAGU_46	F-GCACTCGATTTCAGAGCTGC		PET	$(ACTAT)_4$	164-184	DIX	4.00	0.517	0.633	0.429
	R-TTCAAGGATGGATGGCATCG	T7				SL	3.00	0.516	0.533	0.921
						SVC	4.00	0.618	0.478	0.341
						Mean	3.67	0.550	0.548	
CAGU_47	F- TCCCTCAGTCCAACGTC		VIC	$(AGCTCC)_4$	345-381	DIX	5.00	0.387	0.363	0.805
	R-GAACCTTCCTTTGTCCGCAC	CAGT				SL	4.00	0.400	0.575	0.117
						SVC	3.00	0.118	0.452	0.000***
						Mean	4.00	0.302	0.463	
CAGU_48	F-TGCACCCTTAGAGACCATGG		FAM	$(ACAGAT)_4$	406-448	DIX	8.00	0.871	0.763	0.759
	R-GTCAAAGCATCCCGTCCTC	CAGT				SL	5.00	0.692	0.648	0.905
						SVC	6.00	0.686	0.782	0.137
						Mean	6.33	0.750	0.731	
CAGU_50	F-TAGGGAGGGCTTCAGGAAC	CAGT	PET	$(ACAGT)_5$	364-409	DIX	6.00	0.633	0.554	1.000
	R-TAGGTGTCGGCAGGTCTAATG					SL	4.00	0.586	0.691	0.517
						SVC	7.00	0.714	0.738	0.760
						Mean	5.67	0.644	0.661	

[a] Shown are loci names, the forward (F) and reverse (R) primer sequence, the 5' tag used for incorporation of the fluorescent tag M13R (GGAAACAGCTATGACCAT), T7 (GCTAGTTATTGCTCAGCGG) or CAGT (CAGTCGGGCGTCATCA), labeling dye used, repeat motif of the sequenced clone, allele size range in base pairs, the number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) determined as the mean value from 32 individuals of *Calochortus gunnisonii* (CAGU) at Dixon Reservoir (DIX), CO, 32 individuals from Sand Lake (SL), WY, 34 individuals from Shavano Campground (SVC), southern CO, the p-value associated with departure from Hardy–Weinberg Equilibrium (HWE), and the inferred presence of null alleles.

CHAPTER III

THE ROLE OF GLACIAL OSCILLATORY DEMOGRAPHIC CHANGES IN THE CENTRAL AND SOUTHERN ROCKY MOUNTAINS IN SHAPING THE GENETIC STRUCTURE OF GUNNISON'S SEGO LILY

Introduction

A fundamental aspect of population genetic structure is the portioning of genetic variation within and among populations (Wright, 1965). The accelerated pace of molecular biology and bioinformatics has improved the understanding of landscape patterns of genetic variation in organismal populations and the ability to infer processes of population establishment and divergence. Avise (1987) suggested that long-term isolation of populations should tend to structure the architecture of species in a geographically concordant manner. Glaciers of the Pleistocene have expanded and contracted population ranges leading to contemporary genetic structure for organisms around the globe (Taberlet, 1998; Hewitt, 2004). Indeed multiple studies support this view in western North America. For example, populations of plants in the Pacific Northwest display distinct genetic genotypes in the northern and southern portions of their ranges, with distinct glacier barriers at the sympatry zone (reviewed in Soltis et al., 1997; Noonan, 2001). During the last glacial maximum (LGM), the Cordilleran and

Laurentide ice sheets covered much of the northern Rockies into northern Montana. The central Rockies were covered in a roughly 1000m thick layer of ice (Pierce, 1979) and the southern Rockies were characterized by less continuous, more localized montane glaciers (Elias, 1996; Leonard, 2007). Topography and climate are heterogeneous across the Rocky Mountain cordillera and extant population structures may signal episodic population contraction and expansion characteristics for individual species mirroring that of glacial cycles across the Pleistocene and Holocene epochs (DeChaine and Martin, 2005; Funk et al., 2005; Hewitt, 2004). Partitioning of genetic variation has also been found in the Rocky Mountains directly related to glacier cycles such as *Boechera holboelli* (Dobes et al., 2004), *B. stricta* (Song et al., 2006), *Sedum lanceolatum* (DeChaine and Martin, 2005) and spotted frogs (Green et al., 1996). Pleistocene glaciation has played a large role in shaping genetic variation patterns evidenced by similar geographic patterns among many western North American species (Soltis et al., 1997; Song et al., 2006).

During glacial periods organisms survived in ice-free patches within high alpine nunataks, the interior of mountain systems, or on the periphery of mountain systems. These ice-free areas are known as refugia (Hewitt, 2004; Holderegger and Thiel-Egenter, 2009; Rull, 2009). Existence of refugia and the dynamics of postglacial recolonization can be investigated through the distribution patterns of genetic variation within and among populations. Two scenarios are evidenced for genetic partitioning within the Rocky Mountain Cordillera: 1) populations are divided into distinct southern and northern genotypes, with northern genotypes having less genetic diversity (reviewed in Soltis et al., 1997) 2) populations are partitioned into high diversity ‘pockets’ across the

species' range with no distinct loss of diversity attributed to increasing latitude (DeChaine and Martin, 2005). Two hypotheses have been proposed to interpret these genotype distribution patterns. First, the "Leading Edge Hypothesis" suggests that the succession of long distance founder events during postglacial recolonization results in a decline in intraspecific diversity away from a southern refugium or a set of southern refugia (Lodgepole pine, Cwynar and MacDonald, 1987; spotted frogs, Green et al., 1996; *Boechera holboelli*, Dobes et al., 2004). Second, the "North-South Hypothesis" suggests that several genetically distinct glacial refugia existed both in northern and southern parts of a species range (Douglas fir, Li and Adams, 1989; *Sedum lanceolatum*, DeChaine and Martin 2005; *Boechera stricta*, Song et al., 2006). A number of factors must be considered when applying these hypotheses to organisms within a region. For example, habitat restrictions and/or reproductive strategy (i.e., long-distance dispersal) in *Pinus spp.* (Cwynar and MacDonald, 1987) versus creeping edges of *Calochortus spp.* (Henss, et al., 2013)) can impact the dispersability of organisms across long distances. Patterns of glaciation within the region of interest may also be of value when inferring recolonization pathways or processes (i.e., heterogeneous montane glaciers in the southern Rocky Mountains versus the large Laurentide ice sheet in northern North America). Finally, geographic barriers to gene flow can greatly inhibit recolonization efforts of species and further partition genetic architecture (i.e., intermontane basins).

This chapter investigates the possibility of genetic structure resulting from glacier expansion and contraction in ranges inhabited by *Calochortus gunnisonii* in the central and southern Rocky Mountains. *Calochortus gunnisonii* S. Watson (Liliaceae) has a large distribution encompassing northeastern Arizona, northern New Mexico, much of

Colorado, eastern Utah, large portions of Wyoming, southern Montana, and western South Dakota (Fiedler and Zebell, 2012). *Calochortus gunnisonii* is believed to have colonized the Rocky Mountain range within the last 7 million years and has since expanded to one of the largest distributions within the genus (Fiedler and Zebell, 2012; Patterson and Givnish, 2002). Interestingly, *C. gunnisonii* reaches higher altitudes than a majority of the genus in the southern portion of the Rocky Mountains between 1200-3300 meters (3400-11,000 feet), and the highest within the Great Basin clade (Fiedler and Zebell, 2012). Glacial oscillations may have had profound effects on the genetic structure of this plant, as populations could have been displaced from montane valley to montane valley, using available refugia to reach contemporary range dynamics.

The abundance of diversity in the genus *Calochortus* suggests a dynamic genetic history related to geological (i.e. mountain uplift) and climatological (i.e., glaciation) changes in western North America. Novel, species-specific microsatellite markers have been designed to analyze the genetic architecture of *C. gunnisonii* individuals and populations across multiple mountain ranges in Wyoming, South Dakota, and Colorado. Nuclear markers, such as microsatellites, are biparentally inherited and allow for recombination, thus integrating multiple genealogical processes (Heuertz et al., 2004). The highly polymorphic nature, codominant transmission, ease of detection by polymerase chain reaction, relative abundance, and large genome coverage of microsatellites have resulted in a wide usage both in plants and animals for population genetics and demographic histories (Friar et al., 2007; Kalia et al., 2011; Schwabe et al., 2014; Song et al., 2006; Sugawara et al., 2015; Tucker et al., 2014). By investigating patterns of recent genetic diversification, the role of oscillatory glaciation was assessed in

molding patterns of recolonization and potential areas of refugia for *Calochortus gunnisonii* in the central and southern Rocky Mountains.

Methods

Population Sampling And DNA Isolation

A total of 678 individuals were field collected during late summer 2013 and 2014. Twenty-five populations were carefully selected to represent the mountain ranges spanning the fringes of the Wyoming Basin and a portion of the southern populations in *Calochortus gunnisonii*'s range (Figure 3; Table 3). Herbaria records of <30 years of age were used to find potential geographic areas for sampling (SEINet; Figure 2, Chapter 1). A total of 25 populations were sampled: six populations from the Black Hills, three from the Big Horn range, one from the Absaroka mountains, two from the Sierra Madre range, three from the Medicine Bow mountains, four from the Laramie Mountains, one from the Front Range, one from the Pawnee National Grasslands, and four from central Colorado (Figure 3; Table 3). A total of 2-3 inches of leaf tissue was removed from each individual and stored at -20°C until DNA isolation.

Genetic Analyses

Descriptive Statistics. A total of 678 individuals were analyzed across 13 microsatellite loci according to methods reported in chapter two. Sixty-two individuals were removed from downstream analyses due to a lack of consistent amplification, resulting in a final sample size of 616 individuals. The Burgess Junction (BJ) and County Road 115 (CR115) populations were excluded from most statistical analyses due to the inflation effects of small sampling sizes (i.e., diversity levels). Furthermore, small samples can lead to patterns within the data that do not exist, particularly in genetic

clustering. The GENALEXv6.0.1 plug-in (Peakall and Smouse, 2012) was used to characterize populations by the number of observed alleles (N_O), number of effective alleles (N_E), observed (H_O) and expected (H_E) heterozygosity, and inbreeding coefficients (F_{IS}). A pairwise genetic distance table using F_{ST} between mountain ranges and a Principle Coordinate Analysis (PCoA) were also derived in the GENALEXv.6.0.1 plug-in (Peakall and Smouse, 2012). The number of effective migrants (N_em) between mountain ranges was calculated using the formula from Wright (1951) $N_em \approx \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right)$. Average F_{ST} values were calculated by pooling populations from their respective mountain ranges into a pair-wise table and these values were then used to calculate N_em and placed into a pair-wise format. Linkage disequilibrium between loci was calculated in GENEPOPonline (Rousset, 2008). Analyses of Molecular Variance (AMOVA) were used to assess genetic differentiation within and among populations, regions, and mountain ranges using ARLEQUIN v.3.5.1.2 (Excoffier et al., 2005). AMOVA was also used to assess genetic distribution between the Sierra Madre populations (DCR and SCR) and the rest of the sampling range.

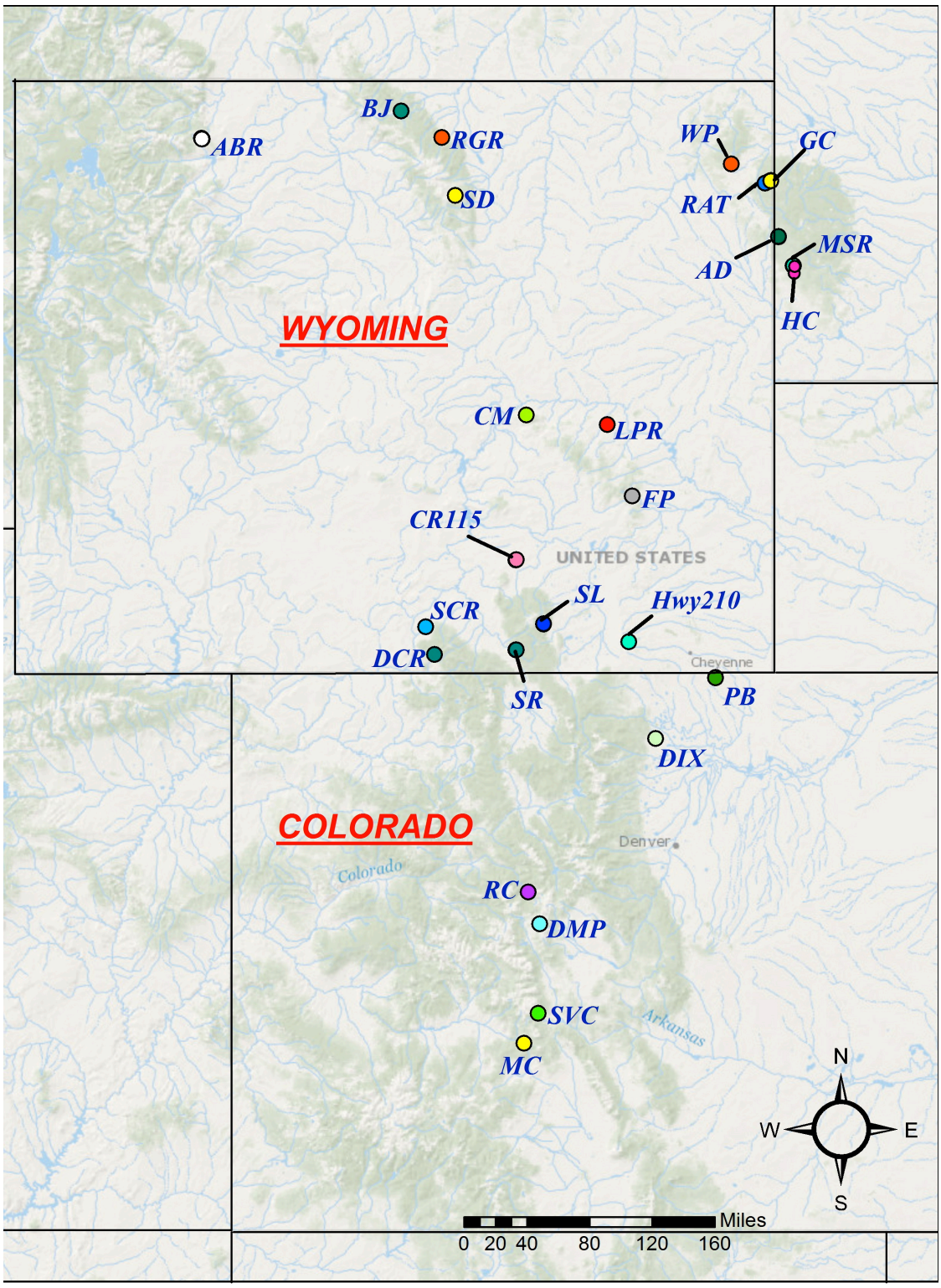


Figure 3: Population sampling map of *C. gunnisonii*.

Table 3. Description and sample size for 25 natural populations of *C. gunnisonii*.

Population	Pop. ID	Mountain Range	Latitude	Longitude	Elevation	n
Absaroka Range	ABR	Absaroka	44.61790029	-109.3231398	8138 ft.	32
Antelope Draw	AD	Black Hills	43.96891917	-104.0005927	6433 ft.	31
Hell Canyon	HC	Black Hills	43.7246205	-103.8540762	5517 ft.	35
Burgess Junction	BJ	Big Horn	44.79731478	-107.4824325	7698 ft.	1
Casper Mountain	CM	Laramie	42.77221617	-106.3269163	6362 ft.	13
County Road 115	CR115	Laramie	41.78337967	-106.4178645	7309 ft.	2
Deep Creek Road	DCR	Sierra Madre	41.12792083	-107.1730007	8043 ft.	31
Dixon Reservoir	DIX	Front Range	40.54135329	-105.1343894	5242 ft.	32
Dyer Mountain Pass	DMP	Central Colorado	39.22815955	-106.2016168	11485 ft.	31
Fletcher Park	FP	Laramie	42.22140717	-105.3482782	7108 ft.	32
Gundinger Spring	GC	Black Hills	44.33875588	-104.0687101	6197 ft.	5
La Prele Reservoir	LPR	Laramie	42.70679583	-105.5785312	5691 ft.	28
Highway 210	Hwy210	Laramie	41.21575567	-105.3822032	8235 ft.	27
Marshall Creek	MC	Central Colorado	38.370582	-106.347185	8972 ft.	30
Mud Springs Road	MSR	Black Hills	43.77463083	-103.8625892	5804 ft.	28
Pawnee Buttes	PB	Front Range	40.9668535	-104.5792528	5715 ft.	30
Rattlesnake Canyon	RAT	Black Hills	44.33145089	-104.0912141	5839 ft.	25
Resolution Creek	RC	Central Colorado	39.45433219	-106.3083899	9603 ft.	13
Red Grade Road	RGR	Big Horn	44.62246683	-107.1067203	7146 ft.	22
Sage Creek Road	SCR	Sierra Madre	41.31924583	-107.2541977	7809ft.	30
Sourdough	SD	Big Horn	44.2406853	-106.9827191	8244 ft.	28
Sand Lake	SL	Medicine Bow	41.33943317	-106.1658852	8904 ft..	32
Savage Run	SR	Medicine Bow	41.16102133	-106.420785	8450	32
Shavano Camp	SVC	Central Colorado	38.586457	-106.215402	9348 ft.	34
Warren's Peak	WP	Black Hills	44.44955623	-104.4348823	6320	12
				Averages		**24.64**
				Total		**616**

*Population names, Pop. I.D. = population code used in study, geographic coordinates (WGS 1984 coordinate system), and n = number of individuals used in microsatellite analysis.

Clustering Analyses. Two Bayesian model-based clustering algorithms were applied to infer population structure and probabilistically assign individuals to genetic clusters without prior knowledge of population units and limits. However, of all of the analyses explained below, only STRUCTUREv2.3.4 used all 25 populations sampled, including the small populations at Burgess Junction (BJ) and County Road 115 (CR115). The first algorithm, STRUCTURE (Pritchard et al., 2000), implements model-based clustering methodology in order to identify potential population structure, assign individuals to populations, migrants, and admixed individuals. The estimation flow-through includes running trial values of the number of *K* populations and then comparing the estimated log probability of data (Ln [Pr(X|K)]) under each *K* value (Fontaine et al., 2007). A series of independent runs were completed with various values of K, testing all values from 1 to 26. Each run was completed under the admixture model with a burn-in of 100,000 iterations and 100,000 iterations of data collection. Ten replicates for each *K*-value were used and checked for consistency of results using the Evanno Method (Evanno et al., 2005) in the web-based software STRUCTURE HARVESTER (Earl and Vonholdt, 2012). The Evanno method assesses the rates of change of log probabilities as the analysis progresses through successive *K*-values (ΔK) and suggests the best *K*-value based on runs displaying the greatest change (Evanno et al., 2005).

A second algorithm, GENELANDv.4.0.3 (Guillot et al., 2005), differs from STRUCTURE by taking into account the spatial dependence of individuals expected for species whose range is much larger than the average intergeneration movement of individuals. The analysis applies individual geographic coordinate data with multilocus genotypes, grouping sampled populations to locate genetic discontinuities between

populations in space. All parameters (including *K* and F_{ST}) are co-estimated simultaneously by Markov Chain Monte Carlo (MCMC) simulations, and posterior probabilities of genetic boundaries between populations and population membership are mapped (Fontaine et al., 2007; Guillot et al., 2005). The analysis was run with the following parameters: *ploidy = diploid*, *620 nuclei*, *population maximum = 23*, *iterations = 1 x* 10^6, *spatial model = TRUE*, *null allele model = FALSE*, *multiple independent runs = NO*. Samples were thinned every 1000 iterations and a post-process burn-in of 250 was used.

Evidence of recent migration rates between populations was assessed using the Bayesian multilocus genotyping procedure implemented with MCMC methods in BAYESASSv.1.3 (Wilson and Rannala, 2003). Estimates of asymmetrical gene flow between populations are represented by the percentage of shared alleles. This analysis does not require populations to be in migration-drift or Hardy-Weinberg equilibrium (Fontaine et al., 2007). The MCMC analysis was run for a total of 10 x 10^6 iterations, with 2.5 x 10^6 discarded as a burn-in to allow the chain to reach stationarity. Samples were thinned every 1000 iterations to infer posterior probability distributions of parameters of interest and viewed in TRACERv.1.6 (Rambaut, 2014) to ensure that stationarity had been reached.

Spatial genetic structure was also investigated by testing for isolation by distance (IBD). Geographic distances between populations were calculated in a pair-wise fashion via ARCMAPv.10.2.2 (ESRI, Redlands, California). A Mantel test with 10,000 random permutations was performed between populations comparing the Rousset distance ($F_{ST}/(1-F_{ST})$; Rousset, 1997), the matrix of pairwise genetic differentiation, and

geographic distance between populations (kilometers). The analyses were performed with IBDws (Jensen et al., 2005) on all 23 populations and a separate analysis excluding the Sierra Madre cluster was also run after identifying a particularly high F_{ST} between neighboring mountain ranges.

Finally, a network analysis of population relatedness using F_{ST} distances was performed in EDENetworks (Kivela et al., 2013). Genetic network analysis can be used to illustrate and understand the history of contemporary patterns of gene flow. The method is a "divisive-hierarchical" clustering-like process in which the network is scanned from a fully connected (percolation) state and the threshold distance is lowered to observe the emergence of clusters at different values. Here, the percolation value was established by the software and used as a starting point and user-defined exploration was used to scan the network across different thresholds. This method allows the user to test the stability of the pattern revealed at the percolation value and reveal the emergence of clusters within nonrandom data sets across different genetic distances (Kivela et al., 2013). This illustrates the different levels of gene flow across different spatial and temporal scales, and possibly biogeographical regions with variable times and levels of divergence. The thresholds used in this analysis were 0.0525, 0.0600, 0.0723 (percolation), and 0.1000. Furthermore, an auxiliary, post-processing file consisting of geographic points for each population was uploaded to the software. This file allows the network to map sampled populations to geographic locations and provide illustration of gene flow across large landscapes.

Results

Descriptive Statistics

Variation Across Loci. A total of 13 microsatellite loci were used to analyze 616 individuals from 25 populations of *Calochortus gunnisonii* (Table 4). The levels of genetic variation including number of alleles (N_A), number of effective alleles (N_E), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) are shown in Table 4 for each locus. The mean $N_A = 3.990$ and $N_E = 2.212$. Marker CAGU_39 displayed the highest number of alleles at 5.000 and CAGU _46 was the lowest at 3.043. All loci showed significant ($p<0.05$) deviation from HWE in at least 3 populations. Pairwise linkage disequilibrium among loci reported significant disequilibrium for CAGU_50, 39, 45, and 48. However, no consistent patterns showed upon manual observation and the disequilibrium was determined to have no effect on downstream analyses. Disequilibrium and HWE deviation was attributed to population level processes, a large geographic sampling range isolating populations, and relatively small sample sizes.

Variation Across Populations. The number of individuals sampled per population varied from 1 to 35 with an average of 24.64 (Table 5). The N_A varied from 2.232 to 4.769 (mean = 2.212) and N_e varied from 1.652 to 3.061 (mean = 3.990). The H_O ranged from 0.257 to 0.465 (mean = 0.369), H_E ranged from 0.330 to 0.636 (mean = 0.493), and F_{IS} ranged from -0.074 to 0.414 (mean = 0.203) across all populations. Observed heterozygosity was lower than H_E across all populations and loci except for Gundinger Spring and this is most likely due to the small population sample (n=5).

Table 4. Genetic statistics of 13 *C. gunnisonii* microsatellite loci averaged over 25 sampled populations

Locus I.D.	N_A	N_E	H_O	H_E	F_{IS}
CAGU_14	3.130	1.985	0.356	0.463	0.214
CAGU_15	3.522	1.960	0.438	0.459	0.046
CAGU_22	3.783	1.947	0.374	0.448	0.168
CAGU_31	4.304	2.438	0.496	0.554	0.110
CAGU_35	3.957	1.939	0.205	0.407	0.469
CAGU_36	3.913	2.114	0.265	0.499	0.448
CAGU_39	5.000	2.910	0.573	0.647	0.111
CAGU_42	3.870	1.931	0.205	0.398	0.420
CAGU_45	3.870	2.196	0.369	0.470	0.188
CAGU_46	3.043	1.724	0.369	0.377	0.017
CAGU_47	3.696	1.932	0.265	0.437	0.365
CAGU_48	5.087	2.809	0.518	0.600	0.120
CAGU_50	4.696	2.873	0.508	0.615	0.162
Totals:	**3.990**	**2.212**	**0.380**	**0.490**	**0.218**

*Number of alleles (N_A), number of effective alleles (N_E), observed heterozygosity, expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}). CAGU = *Calochortus gunnisonii*.

Table 5. Population level genetic statistics of 25 populations of *Calochortus gunnisonii*.

Population	Mtn. Range	*n*	N_A	N_E	H_O	H_E	F_{IS}
Absaroka (ABR)	Absaroka Range	32	3.000	1.652	0.295	0.330	0.101
	Average	***32***	3.000	1.652	0.295	0.330	0.101
Burgess Junction (BJ)	Big Horn Mtns.	1.0	-	-	-	-	-
Red Grade Road (RGR)	Big Horn Mtns.	22	3.462	1.983	0.322	0.448	0.219
Sour Dough (SD)	Big Horn Mtns.	28	3.615	2.069	0.298	0.459	0.346
	Average	***25.5***	***3.539***	***2.026***	***0.310***	***0.454***	***0.283***
Antelope Draw (AD)	Black Hills	31	3.230	1.796	0.352	0.386	0.075
Mud Springs Rd. (MSR)	Black Hills	28	3.385	1.679	0.315	0.371	0.136
Warren's Peak (WP)	Black Hills	12	2.846	2.036	0.283	0.446	0.367
Rattlesnake Canyon (RAT)	Black Hills	25	3.462	2.061	0.261	0.460	0.414
Gundinger Spring (GC)	Black Hills	5	2.231	1.798	0.444	0.395	-0.074
Hell Canyon (HC)	Black Hills	35	3.462	2.025	0.425	0.497	0.149
	Average	***22.7***	***3.102***	***1.900***	***0.347***	***0.426***	***0.178***
Casper Mountain (CM)	Laramie Mtns.	13	3.769	2.305	0.457	0.501	0.068
La Prele Reservoir (LPR)	Laramie Mtns.	28	4.231	2.233	0.422	0.503	0.160
Fletcher Park (FP)	Laramie Mtns.	32	4.538	2.332	0.444	0.548	0.210
Highway 210 (Hwy210)	Laramie Mtns.	27	4.769	2.126	0.425	0.497	0.149
	Average	***25***	***4.326***	***2.249***	***0.437***	***0.512***	***0.147***
County Road 115 (CR115)	Medicine Bow Mtns.	2	-	-	-	-	-
Sand Lake (SL)	Medicine Bow Mtns.	32	4.231	2.269	0.465	0.518	0.127
Savage Run (SR)	Medicine Bow Mtns.	32	4.615	2.417	0.419	0.560	0.232
	Average	***32***	***4.423***	***2.343***	***0.442***	***0.539***	***0.180***
Deep Creek Road (DCR)	Sierra Madre Mtns.	31	3.846	1.839	0.300	0.426	0.259
Sage Creek Road (SCR)	Sierra Madre Mtns.	30	3.770	2.100	0.257	0.457	0.346
	Average	***30.5***	***3.808***	***1.970***	***0.279***	***0.442***	***0.303***
Dixon Reservoir (DIX)	Front Range	32	5.923	2.521	0.458	0.562	0.182
Pawnee Buttes (PB)	Front Range	30	4.462	2.431	0.465	0.559	0.185
	Average	***31***	***5.193***	***2.476***	***0.462***	***0.561***	***0.184***
Shavano Camp (SVC)	Central CO Rockies	34	4.692	2.575	0.463	0.573	0.183
Resolution Creek (RC)	Central CO Rockies	13	4.308	2.914	0.437	0.620	0.291
Dyer Mountain Pass (DMP)	Central CO Rockies	31	4.615	2.660	0.418	0.584	0.268
Marshall Creek (MC)	Central CO Rockies	30	5.308	3.061	0.458	0.636	0.284
	Average	***27***	***4.731***	***2.803***	***0.444***	***0.603***	***0.257***
	Global Mean		3.990	2.212	0.369	0.493	0.203

*Population, population code used in study, mountain range, number of alleles (N_A), effective alleles (N_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}). CR115 and BJ not reported due to low sampling number (n=2 and 1 respectively).

Population pairwise F_{ST} was used to evaluate genetic differentiation between populations. Average mountain range pairwise F_{ST} comparisons were calculated for the Black Hills, Big Horn, Laramie, Medicine Bow, Sierra Madre, Northern Colorado, Central Colorado, and Absaroka ranges (Table 6). The lowest F_{ST} values were found along the diagonal where ranges were compared. The highest F_{ST} value was at 0.301 between the Absaroka and Sierra Madre mountain ranges.

Table 6. Pair-wise genetic distance table (F_{ST}) between sampled mountain ranges for *C. gunnisonii*.

RANGE	*Black Hills*	*Big Horn*	*Laramie*	*Medicine Bow*	*Sierra Madre*	*Central Colorado*	*Front Range*	*Absaroka*
Black Hills	0.056							
Big Horn	0.197	0.026						
Laramie	0.104	0.047	0.020					
Medicine Bow	0.160	0.108	0.070	0.033				
Sierra Madre	0.256	0.205	0.171	0.058	0.033			
Central Colorado	0.167	0.126	0.097	0.054	0.090	0.044		
Front Range	0.106	0.048	0.027	0.070	0.165	0.077	0.020	
Absaroka	0.165	0.136	0.163	0.196	0.301	0.19	0.139	

*All F_{ST} values were significant ($p<0.05$) for each pairwise comparison (data not shown).

All individuals and populations were analyzed for hierarchical analysis of molecular variance (AMOVA) (Table 7). A majority of the variation was found within populations (81.65%), while 18.35% of the variation was found among populations ($p<0.0001$; Table 7). When the AMOVA was applied to reflect the number and membership of clusters found in preliminary STRUCTURE results (K=5: Black Hills; Absaroka Range; Sierra Madre and Medicine Bow; Big Horn Range, Laramie Range and Front Range; central Colorado) a majority of the variation was found within populations

(78.81%), 4.35% was partitioned among populations within ranges, and 16.85% was partitioned among ranges ($p<0.0001$; Table 8). A final AMOVA analyzed the Sierra Madre range against the rest of the sampled range. A total of 70.99% of the variation was partitioned within populations, 12.93% was partitioned among populations within groups, and 16.08% of the variation was partitioned between the Sierra Madre populations and the rest of the sampled range ($p<0.0001$; Table 9).

Table 7. AMOVA results testing genetic subdivision among all Individuals and populations.

All Individuals and Pops	**Source of Variation**	**d.f.**	**Sum of Squares**	**Variance Components**	**% of Variation**
F_{ST} = 0.18350	Among Populations	22	828.663	0.65462	18.35
P = <0.0001	Within Populations	1203	3503.962	2.91269	81.65
	Total	1225	4332.625	2.597	

Table 8. AMOVA results testing genetic subdivision among clusters identified in STRUCTURE.

Between Five STRUCTURE Groups	Among Ranges	4	626.962	0.62268	16.85
F_{ST} = 0.21193	Among Populations within Clusters	18	201.701	0.16060	4.35
P = <0.0001	Within Populations	1203	3503.962	2.91269	78.81
	Total	1225	4332.625	3.69597	

Table 9. AMOVA results testing genetic subdivision between the Sierra Madre and Global Population.

Sierra Madre vs. Global Pops	Among Groups	1	180.063	0.659746	16.08
F_{ST} = 0.29010	Among Populations within Groups	21	648.600	0.53051	12.93
P = <0.0001	Within Populations	1203	3503.962	2.91269	70.99
	Total	1225	4332.625	4.10293	

A PCoA analysis resulted in the first coordinate explaining 48.07% of the observed variation and effectively split the sampled ranges into a Black Hills, Absaroka, Big Horn, and Laramie group and a central Colorado, Medicine Bow, and Sierra Madre group (Figure 4). The second coordinate explained 18.25% of the variation and further subdivided the Black Hills from the Laramie, Big Horn, and Absaroka group. When examining the entire PCoA, the Black Hills split into a distinct group separate from the global group of populations and ranges. The Big Horn Range (SD and RGR) grouped together with the Absaroka Range. Populations PB and DIX in the Front Range of Colorado were clustered into the Laramie Range grouping. The central Colorado populations were separated by coordinate two and SVC was distinctly removed from the cluster by this axis. The Medicine Bow populations, SL and SR, show a pull between the Sierra Madre and Laramie Ranges, but are largely nested within the central Colorado grouping. The Sierra Madre is distinctly separated from the rest of the global samples by coordinate one and two. Coordinate three resolved 11.26% of the variation and predominantly separated the Absaroka Range into its own distinct group (not shown).

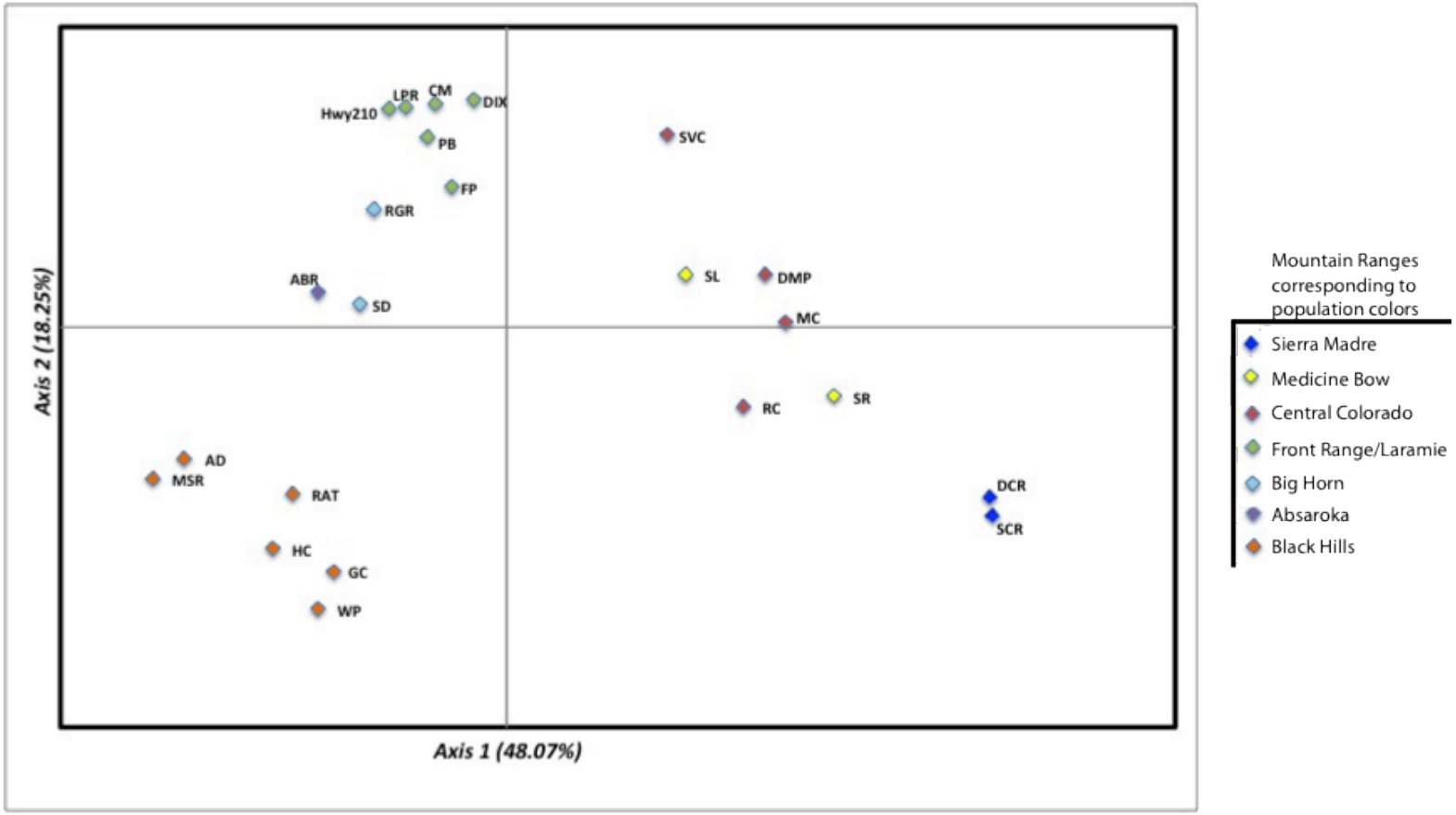


Figure 4: Principle Coordinate Analysis (PCoA) of *C. gunnisonii*. Shown are Axis 1 (48.07%) and Axis 2 (18.25%). The legend in the figure names the Mountain ranges of the correlated colored individuals in the PCoA.

Cluster Analyses. Analysis of posterior probability values in STRUCTURE HARVESTER suggest a *K* of 2 as the most suitable number of genetic clusters for detection of variation across the microsatellite dataset (Figures 5 and 6). A bar graph of this analysis displays distinct separation of the northern mountain ranges (Black Hills, Big Horn, and Absaroka) from the southern ranges (Sierra Madre, Medicine Bow, and Central Colorado Mountains). A region of admixture between the two clusters exists within the Laramie and Front Ranges (Figure 7). Analysis of the Evanno Δ*K* suggests another potentially explanatory *K*-value of 5 (Figure 6). In this scenario, all of the populations display alliance to their geographic range of origin (Figure 8). The Absaroka (blue) and Black Hills (yellow) populations show unique and distinct signals with very little admixture. The Big Horn Range (yellow, purple, and blue) is highly admixed with predominate signal from the Laramie and Black Hills Ranges. The Big Horn populations also show some signal of admixture with the Absaroka Range, suggesting small amounts of contemporary or historical gene flow. The combined Laramie and Front Range cluster (7 populations) contains ~75% of its own signal (purple), with the remaining 25% displaying admixture from all other ranges in the region. The Sierra Madre range is distinct from the rest of the populations in nearby ranges (red), particularly DCR. The Medicine Bow mountain populations (red and purple) share a majority of the 'Sierra Signal,' however a large signal (~25%) can be observed from the Laramie Mountains within the SL population. The central Colorado populations share a unique signal (green) with a small portion of individuals displaying admixture from the 'Sierra Signal.'

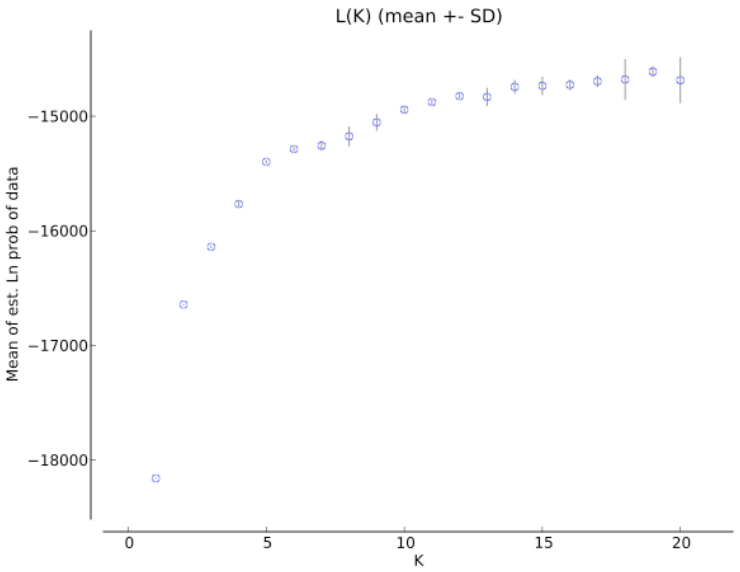


Figure 5: Mean of estimates Ln. probability over 20 K Values

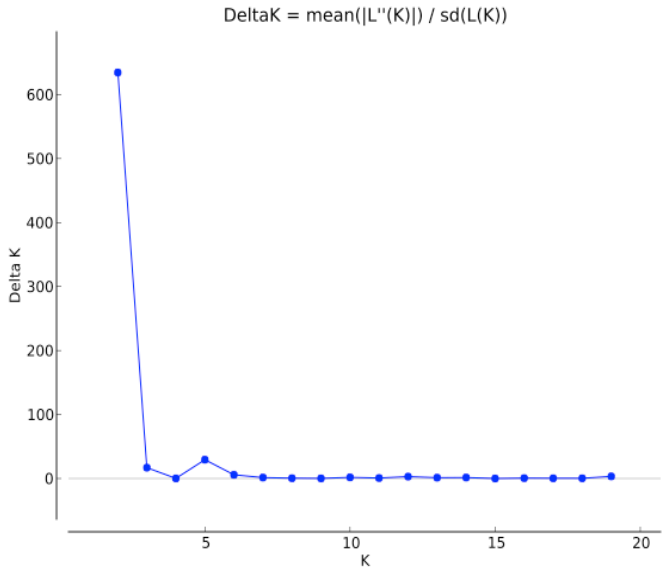


Figure 6: Second-order rate of change (ΔK) of STRUCTURE Likelihood Values

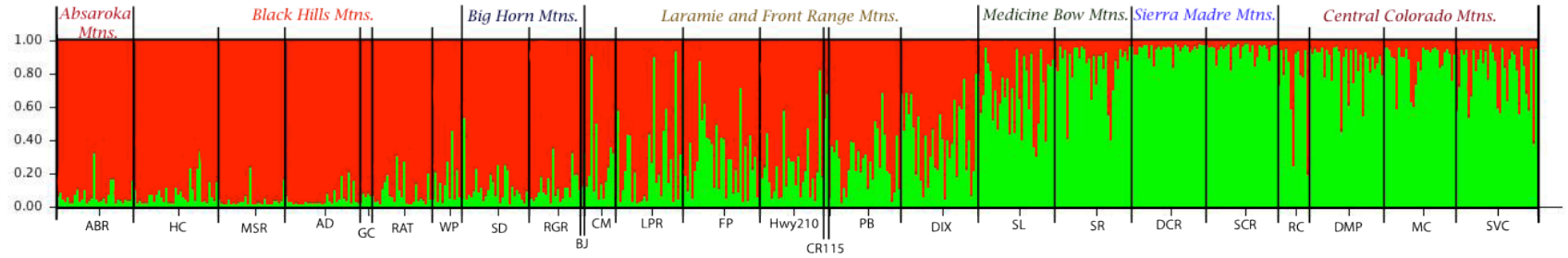


Figure 7: STRUCTURE Bar graph representing a *K*-value of 2. Populations are dictated along bottom axis and the respective mountain ranges on the top axis

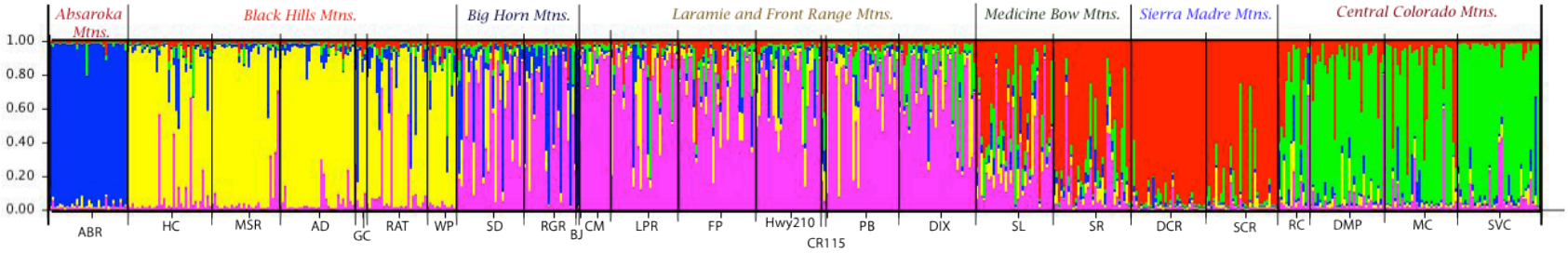
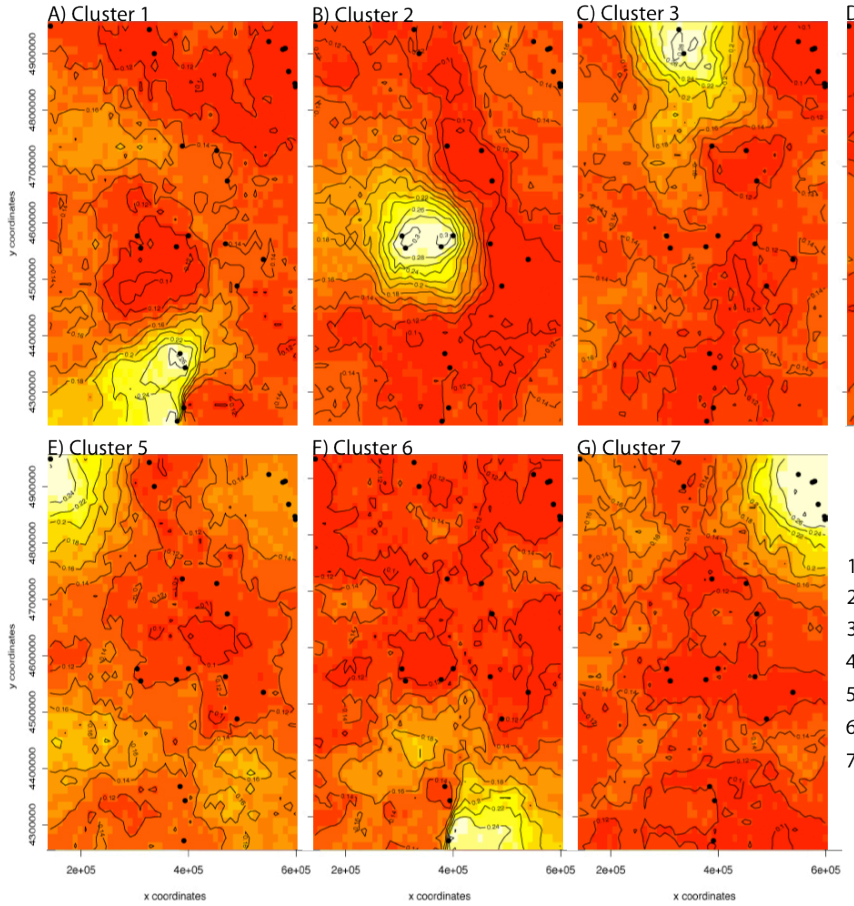


Figure 8: STRUCTURE Bar graph representing a *K*-value of 5. Populations are dictated along bottom axis and the respective mountain ranges on the top axis

GENELAND was used to infer genetic relationships based on individual geographic coordinate data with microsatellite genotypes, grouping sampled populations to locate genetic discontinuities between populations across the sampling region. The software determined that a *K*-value of 7 (i.e., 7 genetic/geographical clusters) showed the most suitable number for detection of meaningful variation (data not shown). A map of the posterior probabilities for genetic discontinuities for all 7 estimated population clusters is shown in Figure 9. Clusters of significant similarity (based on F_{ST} comparisons) are displayed in bright white, decreasing similarity in yellow, and those of least similarity in red. Three central Colorado populations created the first cluster (MC, RC, and DMP). The SVC population in central Colorado was identified as a unique cluster (Cluster 6). The Sierra Signal was found to unite all populations of the Sierra Madre (DCR, SCR) and Medicine Bow Mountains (SR, SL) in Cluster 2. The Big Horn Mountains (RGR, SD) are displayed in Cluster 3. Cluster 4 displays two white 'peaks' uniting Hwy210 of the southern Laramie Range, and PB and DIX of the Front Range to the northern Laramie populations of FP, LPR, and CM. The Absaroka population is displayed in Cluster 6. Finally, Cluster 7 unites the Black Hills populations together as one cohesive unit (HC, MSR, AD, RAT, GC, and WP). Additionally, a pairwise F_{ST} graph was derived by GENELAND. These values ranged from 0.059 between the SVC signal and the MC, RC, and DMP cluster (6 and 1) to 0.335 between the Sierra Madre/Medicine Bow cluster and the Absaroka cluster (5 and 2).



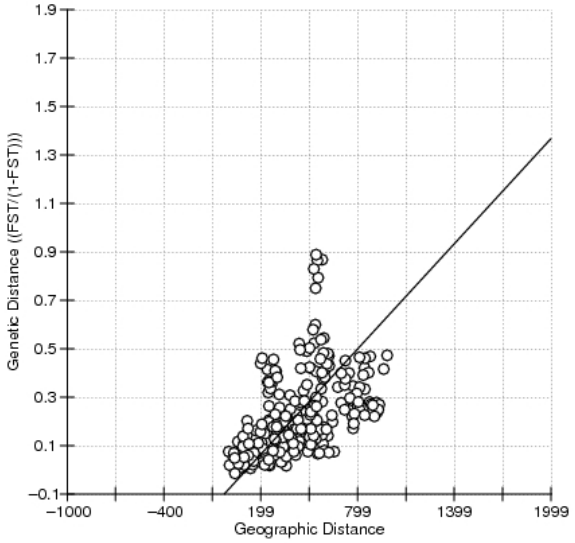
Genetic Distances (Fst) Among Clusters

	1	2	3	4	5	6	7
1	0.000						
2	0.069	0.000					
3	0.169	0.210	0.000				
4	0.129	0.177	0.060	0.000			
5	0.262	0.335	0.245	0.217	0.000		
6	0.059	0.150	0.165	0.107	0.286	0.000	
7	0.217	0.268	0.113	0.120	0.235	0.241	0.000

Figure 9: Map of GENELAND posterior probability assignment of genetic discontinuities of population clusters. Genetic similarities are represented as most similar (white) to least similar (red). Scale axis representing GPS coordinates. F_{ST} values between distinct clusters compare genetic differentiation

Significant correlations between genetic and geographic distances were found using IBD analyses ($p<0.001$). However, the high F_{ST} values correlated with the 'Sierra Signal' decreased the '*r-value*' from 0.6795 (removed Sierra Madre populations) to 0.5022 (all populations) within the Global analysis (Figure 10). For this reason, a second analysis excluding the SCR and DCR populations was completed. The Mantel-test significance values are as follows: a) Global $Z = 29155.5162$, $r = 0.5022$, $R^2 = 0.252$, $p<= 0.001$; b) Sierra populations removed (DCR and SCR) $Z = 21353.8264$, $r = 0.6795$, $R^2 = 0.462$, $p<0.001$.

A)



B)

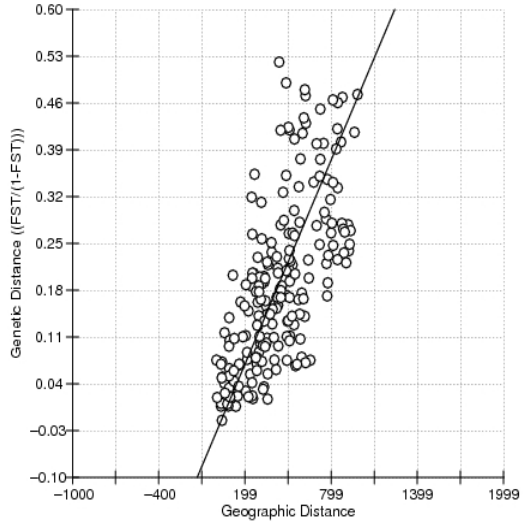


Figure 10: Scatter plots of Rousset's Distance ($F_{ST}/1-F_{ST}$) (Rousset, 1996) versus pairwise geographic distance (kilometers). A) All sampled populations (excludes BJ and CR115), r = 0.5022, $R^2 = 0.252$, $p<0.001$. B) All populations without the Sierra Madre populations (excludes BJ and CR115), r = 0.6795, $R^2 = 0.462$, $p<0.001$.

Evidence of recent migration rates across clusters was assessed using the Bayesian multilocus genotyping procedure implemented in BAYESASSv.1.3 (Wilson and Rannala, 2003). Values are reported as the fraction of individuals in population 1 that are migrants derived from population 2 per generation. Values of >3.00 were considered to be significant for patterns of clustering and admixture seen in other analyses (GENELAND, STRUCTURE). Analysis of the migration rates across mountain ranges did not display a large portion of migrations between ranges. However, the Sierra Madre/Medicine Bow complex had some interesting patterns. The Sierra Madre and Medicine Bow ranges receive a significant amount of the migrants from the SCR population (DCR (20.3), SR (13.4), and SL (~2.75)). The SL population is nearing significance and displays a much higher migration rate than all other non-significant values across the analysis (data not shown). Furthermore, within three range systems a significant 'source' population was identified: 1) within the Laramie Range, LPR was the source population for CM (13.9), DIX (19.0), FP (19.5), Hwy210 (18.9), and PB (19.7); 2) within the Black Hills Range AD was the source population for BH (21.0), GC (14.1), MSR (19.0), RAT (16.7), and WP (13.03); and 3) within the Sierra Madre/Medicine Bow Ranges SCR was the source population for SR (13.4) and DCR (20.3). To supplement this analysis, the number of effective migrants per generation was calculated between mountain ranges using the formula from Wright's statistics (Table 10). The largest values can be observed along the diagonal of the pair-wise comparison where populations within a range are being compared. The Laramie range populations display the largest effective migration rates and share a large proportion of effective migrants with the Big Horn (5.069) and Front Range populations (9.009). The Laramie, Medicine Bow, and Sierra Madre complex

share a high amount of migrants between one another, while the central Colorado, Black Hills, and Absaroka ranges share the highest amounts of migrants with one another (Table 10).

Table 10. Pairwise Number of effective migrants per generation (N_em) between sampled mountain ranges for *C. gunnisonii*.

RANGE	Black Hills	Big Horn	Laramie	Medicine Bow	Sierra Madre	Central Colorado	Front Range	Absaroka
Black Hills	4.214							
Big Horn	1.019	9.365						
Laramie	2.154	5.069	12.250					
Medicine Bow	1.313	2.065	3.321	7.326				
Sierra Madre	0.727	0.970	1.212	4.060	7.326			
Central Colorado	1.247	1.734	2.327	4.380	2.528	5.432		
Northern Colorado	2.108	4.958	9.009	3.321	1.265	2.997	12.250	
Absaroka	1.265	1.588	1.284	1.026	0.581	1.066	1.549	0.000

* N_em calculated using the formula: $$N_e m \approx \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right)$$

Genetic network analysis of the dataset reveals variable degrees of clustering and connectivity across the four tested threshold values (connectivity and distance measures not shown; Figure 11). An initial percolation threshold value (0.07233) (Figure 11C) was generated by EDENetwork, below which the network will begin to show increasing disconnect. At the initial percolation value, mountain range connections reflect STRUCTURE (Figures 7 and 8) and GENELAND (Figure 9) outputs. Furthermore, a few admixed populations evidenced in these genetic clustering analyses are also displayed via connections between proximate populations. When the network threshold is decreased to 0.06 (Figure 11B), most of populations within ranges remain connected, but some connections are lost between ranges. In the Sierra Madre/Medicine Bow complex, a connection between the two ranges remains but connection is lost to both the Laramie and Central Colorado ranges. At the lowest threshold value of 0.0525 (Figure 11A), connectivity continues to decrease. Connectivity among populations within their native mountain ranges remains strong in the Laramie, Black Hills, and Big Horn complexes. The central Colorado complex loses connectivity between all populations except for RC and DMP. The Sierra Madre and Medicine Bow ranges also lost connectivity between populations within their ranges, but conserved a connection between one another (SCR to SR). A manual threshold well above the percolation value was illustrated (Figure 11D) to give potential insights into ancient or contemporary gene flow between clusters. Connections between the Black Hills were established between the Laramie complex and the Big Horn range. Furthermore, additional connections were established between the central Colorado complex and the Medicine Bow range.

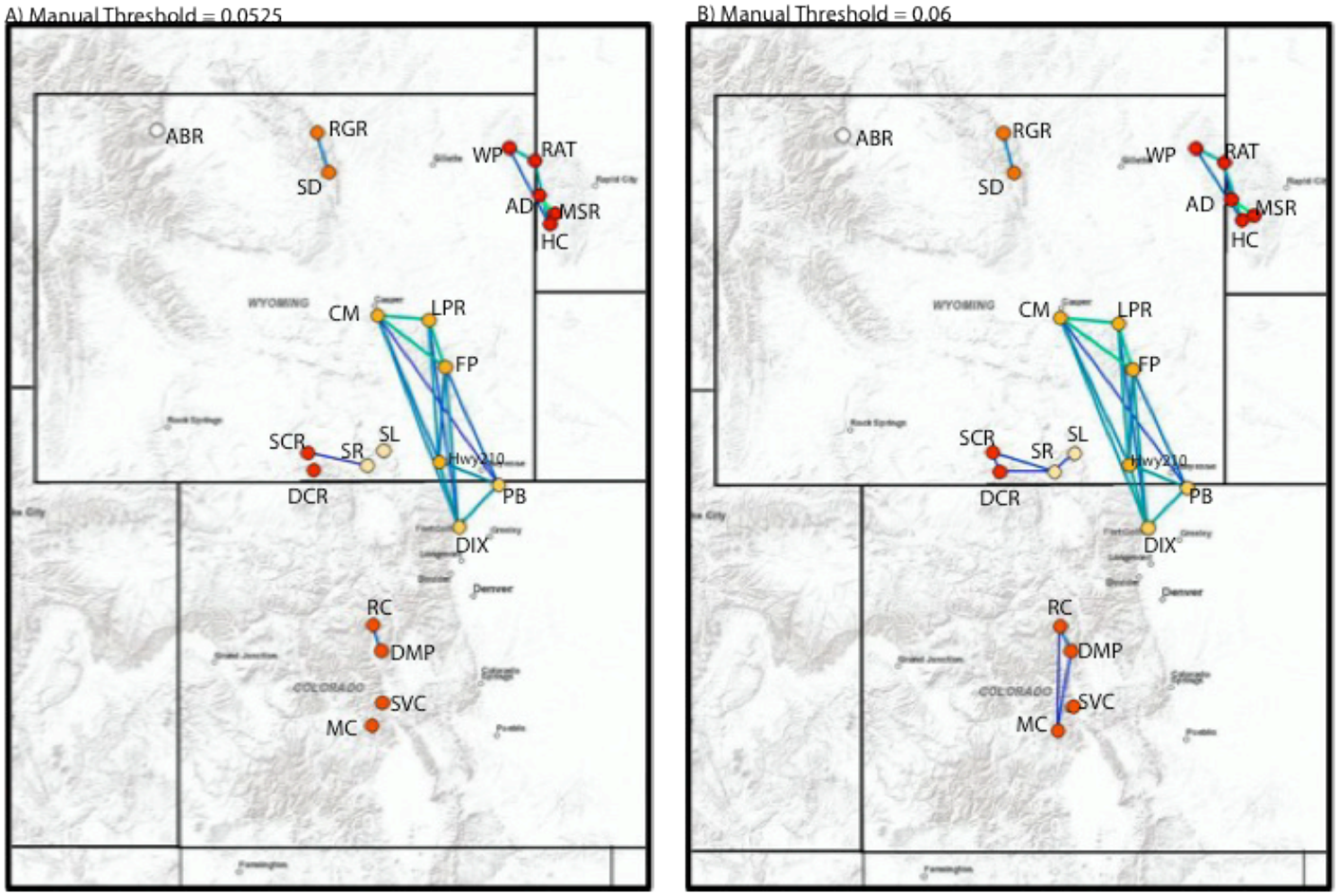


Figure 11: EDENetwork analysis of 22 populations *C. gunnisonii* (GC removed from analysis (n=5)). A) Threshold = 0.0525 B) Threshold = 0.06. Colors and sizes of nodes are fixed.

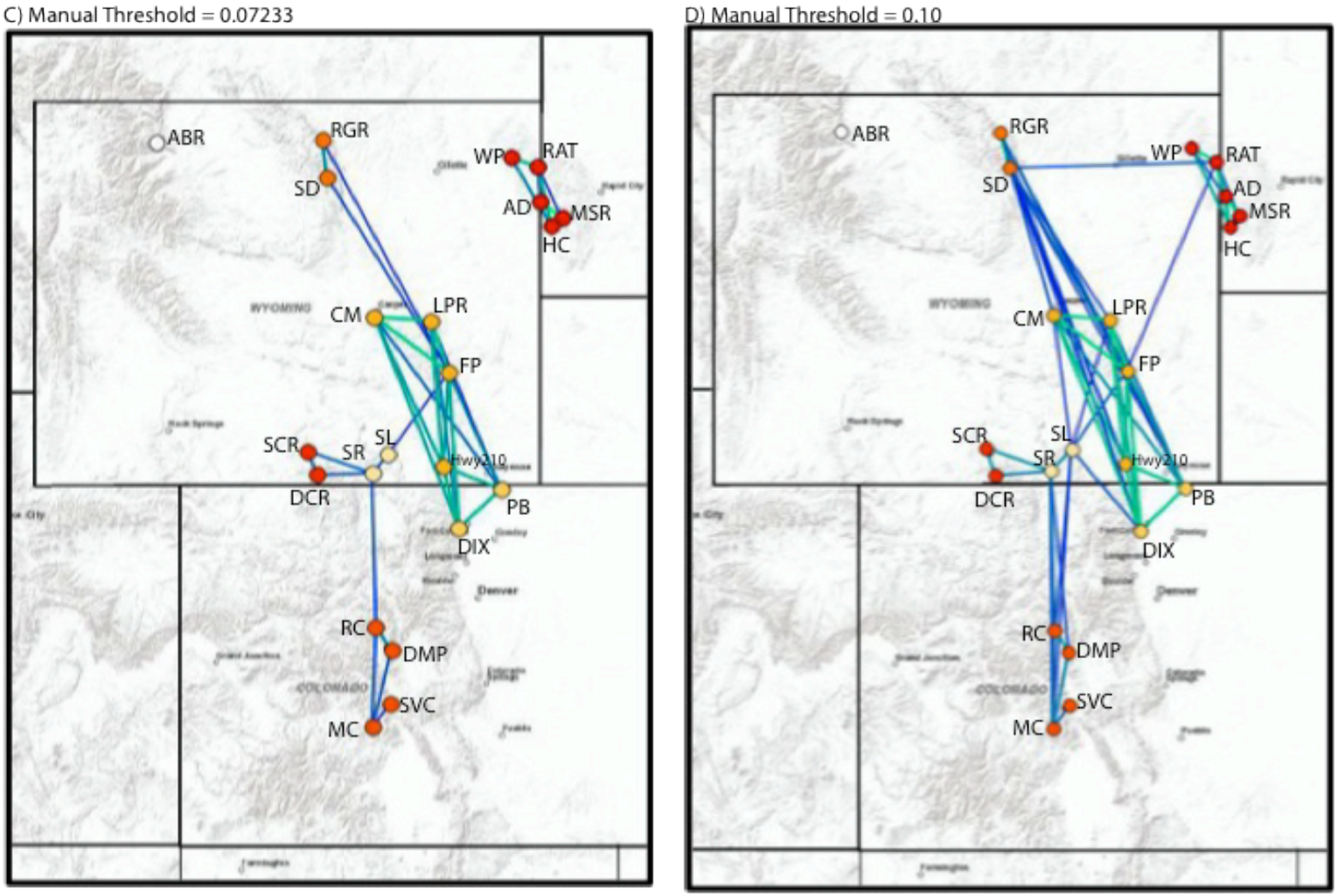


Figure 11: Cont'd from previous page. C) Percolation threshold = 0.07233 and D) displays a threshold of 0.10.

Discussion

Organismal population structure within the Rocky Mountain cordillera has largely been shaped by the climatic oscillations of the Quaternary (Hewitt, 2004; DeChaine and Martin 2004, 2005; Song et al., 2006; Holderegger and Thiel-Egenter, 2009; Rull, 2009; Rebernig et al., 2010). Suitable micro or macro swaths of habitat refugia during glacial periods have been suggested in the central and southern Rocky Mountains for many organisms (Hadley, 1987; Li and Adams, 1989; Mitton et al., 2000; Good and Sullivan, 2001; DeChaine and Martin, 2004,2005; Brunsfeld and Sullivan, 2005; Song et al., 2006). For many of the montane species in this region, the expansion of forest habitat into the basin deserts and grasslands was characterized by 100,000-year cold cycles and 20,000-year warming cycles (Ehlers and Gibbard, 2011). The effects of these range expansions and contractions had profound effects on the genetic structure of many organisms in this region (Song et al., 2006; Brunsfeld and Sullivan, 2005; DeChaine and Martin, 2005; Good and Sullivan, 2001; Li and Adams, 1989; Mitton et al., 2000). Gene flow among populations of *Calochortus gunnisonii* is not equal across all landscapes in the central and southern Rocky Mountain cordillera. Inferred processes that explain distributional patterns of microsatellite genotypes indicate that both contemporary and historic factors shape the biogeography of this lily in the region.

Absaroka Mountains

Previous studies of the Absaroka Range and the mountain chains northward into Montana and Idaho display distinct genetic signal from that of the ranges across the Wyoming Basin (DeChaine and Martin, 2004, 2005; Wilson et al., 2005; Song et al., 2006). The statistical analyses in this study point toward a similar pattern where *C.*

gunnisonii populations in the northern Rockies (Yellowstone area and northward) are distinct from the more southern ranges. The Wyoming Basin is a strong barrier to gene flow of montane organisms due to the combined effects of low altitude and low precipitation (Hadley, 1987; DeChaine and Martin, 2004, 2005; Wilson et al., 2005). Although the southern Rocky Mountains were characterized by more heterogeneous montane glaciations, the Absaroka Range was almost completely covered in ice at the LGM (Leonard, 2007). This would have limited *C. gunnisonii* and other montane species to intermontane or peripheral refugia. Genetic signal from the Absaroka is weak in the Big Horn Range (111 miles across the Big Horn Basin), except for the single Burgess Junction (BJ) sample (Figure 8). This sample represents the most northwestward sampling of the Big Horn range and its ABR signal may be indicative of a northwestern genotype, reflecting admixture from ABR populations across the Big Horn basin. Alternatively, routes of colonization have been suggested for a boreal squirrel species via the Owl Creek Mountains (southern tip of the Big Horns due west to the Absaroka range) or more northerly ranges in Montana (Wilson et al., 2005) and may also have been used as a corridor for gene flow in *C. gunnisonii*. A deeper sampling of this region, especially the western slope of the Big Horn range, is needed to interpret genetic structure, ancestral history, and the potential of suggested contemporary gene flow corridors in this potentially vital bridge area between southern and northern genotypes. The high amount of diversity in the Absaroka population also suggests that the "Leading Edge Hypothesis" did not occur for this region, rather populations recolonized the area from local refugia (North-South hypothesis) (Song et al., 2006).

The 'Sierra Signal' Of The Sierra Madre And Medicine Bow Complex

Glaciation in the Medicine Bow Mountains was dramatic (Means Jr., 2001; Leonard 2007) and may have resulted in montane vegetation dispersal onto the plains and into the nearby Laramie Range, forming what is now the Laramie Complex. Genetic drift and/or local adaptation have molded the differences in detectable genetic architecture between the Sierra Madre Mountain and Laramie Complex. However, populations in the Medicine Bow range share ~25% of their genetic signal with that of the Laramie Complex and ~75% with the Sierra Madre Mountains, suggesting admixture with these ranges during glacial interphases (Figure 8; Figure 4).

The Sierra Madre is unique from the rest of the sampled ranges in this study. Disproportionate migration rates (BAYESASS) were observed from the SCR population of the Sierra Madre to SR of the Medicine Bow and is further supported via GENELAND clustering (Figure 9), EDENetworks (Figure 11) and the $N_e m$ values (Table 10). The Sierra Madre Mountain populations display a unique genotype from that of the nearest neighboring mountain ranges and inclusion of these populations in IBD analyses significantly changes the pattern of IBD across all populations, suggesting the presence of a refugia (Figure 10). An AMOVA with the DCR and SCR populations paired against the global population resulted in a significant amount of variation (16.08%) among groups, and higher than the among populations within group variation (12.93%) (Table 9). Extensive glaciation seems to have isolated populations in western Wyoming desert-associated refugia where population processes such as drift and/or selection have had profound effects on population structure. During the current warming interphase, the

Sierra Madre genetic type has infiltrated the Medicine Bow Range to the east and Central Colorado to the south. Evidence supports the existence of two potential refugia for *C. gunnisonii* in the Laramie (see below) and Sierra/Medicine Bow Complexes, respectively.

Laramie, Big Horn, And Black Hills Complex

The Laramie and Black Hills mountain ranges do not show signs of Pleistocene glaciation (Mears Jr., 2001; Wilson et al., 2005). Here, the Laramie Complex originally contained a four population network defined by sampling origin within the Laramie Mountain Range and did not include the two Front Range populations. However, all statistical analyses identified the DIX and PB populations as non-differentiated members of this group. The newly united cluster was also identified by EDENetworks and resisted fragmentation across all thresholds in the analysis (Figure 11). Furthermore, this complex displays high pairwise FST values when compared to other populations except for the Big Horn (0.047; Table 6) and Medicine Bow Mountains (mean =0.070; Table 6) and clusters together in PCoA (Figure 4). The distinction of this cluster in the southern Rocky Mountains may pertain to a plains macrorefugia, where large levels of diversity persisted through multiple glacier events in an ice-free habitat. The slightly decreased amount of genetic divergence between the Laramie Complex and the Medicine Bow ranges is reflective of similar origins.

Evidence exists for a large boreal forest spanning the Tongue-Powder and Cheyenne-Fourche River basins connecting areas between the Laramie, Black Hills, and Big Horn Ranges until at least 10,500 to 9,650 years ago (Wilson et al., 2005). The Big Horn Range populations display significant similarities to the Laramie Complex across multiple statistical analyses and it can be inferred that the isolation of populations in this

mountain range probably occurred in the recent past (Pairwise F_{ST} = 0.047, Figure 6; $N_e m$ = 5.069, Table 10; EDENetworks, Figure 11 C and D). Although the Black Hills distinctly represent a unique set of microsatellite genotypes, the EDENetwork supports connections to the Laramie Complex at a threshold of 0.10. A weak, but detectable migration rate can also be found between the Laramie Complex and Big Horn and Black Hills ranges. Furthermore, a boreal squirrel species shows significant genetic similarities between that of the Laramie Mountains and the Black Hills, estimating isolation at 8,450 to 4,680 years ago (Turner, 1974; Wilson et al, 2005). Corridors for organisms in this region are evident and isolation was recent; however, contemporary gene flow does not seem sufficient enough to overcome the effects of genetic drift and/or local adaptation. Pairwise F_{ST} values for the Black Hills show that the Laramie Complex is the most closely related set of populations (F_{ST} = 0.104), despite large genetic structure differences (F_{ST} = 0.104). The results here support diffusion of *C. gunnisonii* across the river basins from the Laramie Complex to the Big Horn and Black Hills mountains via boreal forest corridors that once covered the region and during glacial interphases; this region stands as a large, low elevation refuge for *C. gunnisonii*.

Central Colorado Populations

A distinct separation of the central Colorado populations was evidenced by all clustering analyses in this study. Two EDENetwork threshold values displayed a connection between these populations and the refugia of the Sierra Madre region (Figure 11 C and D), potentially evidencing the diffusion of populations northward and reaffirming northward plant expansions during glacial intervals (Soltis et al., 1997; Hewitt, 2004; DeChaine and Martin, 2005; Holderegger and Thiel-Egentor, 2009).

Populations from this region are correlated with moist montane habitat and reach the largest numbers of individuals across the sampling range (DMP population ~500-1000 individuals, pers. obs.). Furthermore, average heterozygosity and F_{IS} in this region is high ($H_O = 0.444$; $F_{IS} = 0.257$). The highest peaks of the southern Rocky Mountains occur in this area and large alpine glaciation events that occurred in the Sawatch Mountains, in the Front Range to the north, and San Juan Mountains to the west, may have acted as barriers for gene flow for extended periods during the Pleistocene, creating geographic-genetic structure in the Colorado region for many organisms (Hafner and Sullivan, 1995, Wilson et al., 2005). Furthermore, extensive glaciation cut from east to west in this region (Leonard, 2007), preventing gene flow between northern and southern populations. The distinct split between these two represents a southern and northern area of refuge for *C. gunnisonii*, similar to a mitochondrial study involving a boreal squirrel species that shares habitat with *C. gunnisonii* (Wilson et al., 2005). The SVC population is unique in this region as evidenced by genetic clustering analysis (Figure 9) and PCoA (Figure 4) and is most likely uniquely structured due to the complex climate history within the geographically heterogeneous area and is most likely derived from a central Colorado refugia or local mountain peak nunatak(s).

North-South Hypothesis

Evidence of long distance dispersal for *C. gunnisonii* is lacking. Reproductive biology predicted this, however, external forces such as animal dispersal also seem unlikely. *Calochortus gunnisonii* contains high amounts of diversity at all sampled populations, suggesting multiple local refugia within the region (i.e., Laramie Complex, Sierra Madre/MedBow Complex, central Colorado, and Absaroka). The "North-South

Hypothesis" suggests that populations of a species reside in multiple local refugia over glacial interphases and expand to recolonize the area in a largely random manner (Song et al., 2006). The highly fragmented partitioning of genetic diversity in *C. gunnisonii* of the southern Rocky Mountains suggests relevancy for the North-South Hypothesis.

Patterns Related To Species Concepts

A primary goal of evolutionary biology is to comprehend the mechanisms that have created species diversity. Peripatric theory states that species arise when small populations become isolated from an ancestral population and differentiate due to no or limited gene flow (Coyne and Orr, 2004). Large fragmentation of *C. gunnisonii* populations via glacier cyclicity in the central and southern Rocky Mountains is apparent. The genetic structure of the central and southern Rockies, molded by expanding and contracting of populations, has created pockets within refugia containing large amounts of genetic diversity and differentiation. Clusters of populations in the study region display peripatric divergence (i.e., Laramie Complex, Sierra Madre/Medicine Bow Complex, central Colorado, and Absaroka); however, the relatively short time spans (from an evolutionary and geological perspective) of expansion and contraction of populations may not be sufficient to promote speciation with drift alone. The biogeographical consequences of the climate changes causes whole ecosystems to shift their range, not just one species as evidenced by multiple organisms sharing similar disjunctions in western North America (Soltis et al., 1997; Noonan, 2001; Song et al., 2006). The shift of *C. gunnisonii* with montane habitats most likely involved the shift of its pollinators (among other potential selective factors) and greatly limited the effects of natural selection. Furthermore, the 'genetic rescue effect' may be prominent within the

region during glacier cycles as mountain range populations migrate to admix (Hadley, 1987).

Summary

Global climatic oscillations were common in the Quaternary (2.4 MY BP), characterized by 100,000 year cooling and 20,000 year warming phases. Large continental ice sheets covered vast portions of Europe and North America. Biogeographers have long hypothesized how high latitude organisms survived during these harsh cycles. The quickly evolving field molecular analyses allowed researchers to investigate populations within areas characterized by high glaciation and uncovered the presence of patchy, suitable habitat within, and on the periphery of, these areas (refugia). Mountain areas are of particular interest due to the heterogeneous nature of their glacier events. Populations of plants show both a latitudinal and admixed pattern of response to the climate cycles. The central and southern Rockies have largely been characterized by a north and south genetic signal divided by the low altitude and low precipitation area of the Wyoming Basin.

Calochortus gunnisonii is highly fragmented by habitat patchiness in the central and southern Rocky Mountains. Genetic analyses confirm a significant amount of genetic divergence between mountain regions related to alpine glacier cycles. A northern Rocky Mountain genetic signal was identified in the Absaroka Range, which underwent harsher glaciation cycles than the more southern ranges. This, in effect, most likely subjected the genetic structure of the northern populations to a much different history in which admixture with more geographically distant populations was less frequent, and genetic diversity was maintained in local refugia.

A plains macrorefugia has been suggested within the Laramie Complex of the Front Range Mountains. This area was absent of glacier activity during the Pleistocene glacier events and represents a diffusion of displaced *C. gunnisonii* populations from more elevated ranges. Furthermore, large boreal forests covered the river basins between the Laramie, Big Horn, and Black Hills Mountains up to ~10,000 years ago, allowing for diffusion from the Laramie complex into these satellite mountain ranges.

The Sierra Madre Signal is distinctly united to the complex climate history of the Sierra Madre and Medicine Bow mountain ranges. Populations in this area were most likely restricted to microrefugia during multiple, harsh glacier events, migrating up- and downslope, and preventing admixture with nearby mountain ranges (northern Colorado Rockies and the Laramie Complex). Remnant genetic signal from the Laramie Complex can be observed in the SL population of the Medicine Bow Range and may suggest that these populations were once diverse and initially founded the Laramie Range Complex (STRUCTURE, EDENetworks and GENELAND). Furthermore, genetic signal of the SCR population (lower elevation) suggests a basin radiation in which admixture occurred between the SR population of the Medicine Bow Mountains, significantly contributing genetic signal of the central Rockies into further down stream founder events (i.e., the Laramie Complex).

The southern Colorado populations possess yet another unique genetic signal. Large peaks in the region contain alpine glaciers that have undoubtedly affected the shape and magnitude of contemporary population ranges for multiple organisms in the region (DeChaine and Martin et al., 2004, 2005; Wilson et al., 2005). Sampled *Calochortus gunnisonii* populations for this study are geographically close to one another

and form a cluster at the genetic level. However, the SVC population shows a distinct signal that separates it relatively quickly under decreasing thresholds and is given its own unique cluster in GENELAND analyses. This is probably due to the heterogeneous landscape of the area where it is characterized by rapid elevation changes and moisture regimes dictating population admixture.

Gunnison's Sego Lily reflects Pleistocene glacier cycles in the southern Rockies at the genetic level and highly fragmented populations exist due to restriction in refugia during the current warming phase. High diversity pockets of population clusters exist in ranges that have undergone extreme glacier history or are near-neighbors to such cases. *Calochortus gunnisonii* reflects a turbulent past in the southern Rocky Mountain region, genetically shaped by climate oscillations of the Quaternary and suggests an importance of multiple ranges as refugia for a diversity of other organisms in the region (i.e., boreal squirrels, Wilson et al., 2005).

CHAPTER IV

SUMMARY OF *CALOCHORTUS GUNNISONII* GENETIC INVESTIGATIONS IN THE CENTRAL AND SOUTHERN ROCKY MOUNTAINS

Background

This thesis presented an investigation into the evolutionary history of Gunnison's Sego Lily, *Calochortus gunnisonii*. The primary research aim of the study was to infer the role of glacial oscillatory demographic changes in the central and southern Rocky Mountains in shaping genetic structure of populations across multiple montane disjunctions. *Calochortus gunnisonii* is characterized as a mountain lily species and currently inhabits a large geographic range within the central and southern Rocky Mountains of western North America from elevations of 1200 to 1300 meters (3400-11,000 ft.). Herbarium records indicate disjunctions stemming from intermontane basins where suitable habitat is either too patchy or absent in the current climate conditions. Isolation of populations to geographic areas via peripatric means submits populations to local genetic drift and/or selection pressures and should reflect genetic architecture in a geographically concordant manner.

Current genetic studies of *Calochortus* are limited to AFLP (Henss et al., 2013) and chloroplast DNA analyses (Patterson and Givnish, 2003). Neutral, codominant markers, such as microsatellites, were lacking for the genus. Here, 13 novel species-

specific microsatellites were designed for analysis of *C. gunnisonii* populations within mountain ranges from Colorado, Wyoming, and South Dakota.

Major Findings

All thirteen microsatellite loci were polymorphic for *Calochortus gunnisonii* and used to analyze 616 individuals spanning seven mountain ranges in the southern Rocky Mountain cordillera. Genetic analyses display large amounts of diversity and structure for each population and region. Patterns of relatedness between regions indicate recent colonization and diversification. Furthermore, genetic clustering of populations suggests that multiple areas within the region have served as macro and microrefugia for *C. gunnisonii* during Pleistocene glacier events. The Laramie Complex, consisting of low elevation, non-glaciated mountains unites as a highly diverse set of populations displaying past and contemporary gene flow. Additionally, the Black Hills Mountains represents the farthest eastward expanse of the southern Rocky Mountains and populations in this area unite as a distinct entity. The Big Horn Range reflects admixture of multiple genotypes, with distinct admixture signal of the Laramie and Black Hills Ranges. Evidence suggests that boreal forest once covered the desert basins of this area between the Big Horn, Laramie and Black Hills Ranges, providing paths for dispersal for multiple organisms. Genetic clustering analyses suggest that the Black Hills has an origin stemming from the Laramie Complex and that population level processes in the current warming interphase are driving current divergence levels.

The Sierra Madre/Medicine Bow complex possesses a distinct signal, classified here as the Sierra Signal, and contains higher diversity levels than nearby mountain neighbors. The distinct signal of the Sierra Madre Range compared to the Laramie

Complex suggests separate origins. However, the Medicine Bow Range carries signal from both entities and may signify the original expansion zone of colonization via western diffusion during severe glaciation at the LGM. The admixture zone of the Medicine Bow Range also suggests past or contemporary gene flow from the east and west.

The central Colorado populations carry a unique southern signal due to the presence of large east/west glaciers of the high peaks in the region. Recolonization to the north and south during glacier expanse, and subsequent isolation has created a distinct central Colorado genotype.

Finally, the Absaroka Range signifies a unique genotype possibly indicative of a northern Rocky Mountain refugium. Isolation of the northern signal from the southern signal is most likely due to the presence of the Wyoming Basin barrier, which also structures the genetic diversity of many other organisms in the region (DeChaine and Martin, 2005; Song et al., 2006).

Calochortus gunnisonii appears to have resided in four montane refugia in the southern Rocky Mountains during warm interphases of the Earth's MV cycles. Long-term isolations result in divergence patterns between refugia and complex patterns of admixture during cooling phases are evident from the genetic data. This lily also inhabits elevations beyond the border of boreal forests on the periphery of mountain ranges. It is this pattern that may be of value as an indicator for recolonization routes of the region for higher alpine floras in future genetic studies.

Future Research

The use of only one genetic marker limits genetic studies to infer past and present population dynamics only using one genealogy. Future work on this plant species should involve the use of population level chloroplast DNA studies. Chloroplast DNA is uniparentally inherited via the maternal plant and can provide paths of seed dispersal. This could be of value in this system in order to confirm pathways of colonization routes and uncover more ancient ancestral histories between refugia.

Sampling of the region was relatively light for this study. Western slopes of the Laramie and Big Horn ranges should be targeted for future studies. Mountain ranges to the north of the Absaroka Range should also be targeted to further assess the genetic architecture and origin of the northern genetic signal detected in this study. The Colorado Rocky Mountains contain a large fraction of herbarium samples (Figure 2) and selective sampling should be carried to investigate the potential for nunatak refugia in higher elevation populations.

Current climate modeling methods are proving to be useful in natural population studies. It is still largely unknown how organisms will respond to warming climates, but this type of modeling can help predict how landscapes will mold to the changes in local climate regimes and thus provide insight into past and future responses of organisms. Glacier modeling has been done in the southern Rocky Mountains (Leonard, 2007) and a combination of this data with climate modeling and diverse genetic marker analyses could shed more insight into past and future population demographics.

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