POPULATION INVENTORY, THREAT ASSESSMENT, AND ANTICIPATED EFFECTS OF GLOBAL CLIMATE CHANGE ON AN ENDEMIC, CLIFF HABITAT SPECIALIST, "ALICIENNA PENSTEMONOIDES" (POLEMONIACEAE)

A Thesis
by
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Department of Biology
POPULATION INVENTORY, THREAT ASSESSMENT, AND ANTICIPATED EFFECTS OF GLOBAL CLIMATE CHANGE ON AN ENDEMIC, CLIFF HABITAT SPECIALIST, *ALICIHELLA PENSTEMONOIDES* (POLEMONIACEAE)

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Abstract

POPULATION INVENTORY, THREAT ASSESSMENT, AND ANTICIPATED EFFECTS OF GLOBAL CLIMATE CHANGE ON AN ENDEMIC, CLIFF HABITAT SPECIALIST, ALICIELLA PENSTEMONOIDES (POLEMONIACEAE)

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The ongoing biodiversity crisis necessitates assessment of rare and endemic taxa so that we can recognize how global climate change influences species distributions and understand when local extirpations may amount to global extinctions. Vertical cliffs provide a unique ecosystem for highly adapted species to avoid competition. Cliff-dwelling species incur strong selective pressures that lead to regional restrictions and narrow distributions. Aliciaella penstemonoides (M.E. Jones) J.M. Porter (Black Canyon Gilia) is a cliff habitat specialist in the Polemoniaceae (Phlox) family and is restricted to vertical cliff habitats above stream and river drainages of the Upper Gunnison River Basin and Headwaters of the Rio Grande in southwest-central Colorado, USA. Field research in 2016 located 14 populations of A. penstemonoides and identified over 2,000 individuals, indicating that this species is locally abundant yet regionally rare. I employed distance sampling line transect methods in combination with rock climbing techniques to document population
density and threats across five populations. MaxEnt software modeled the current extent of suitable habitat and predicted suitable habitat under projected climate scenarios for 2050. Models had excellent accuracy according to evaluation statistics and indicate a loss of 70 - 99% of suitable habitat by 2050. I screened microsatellite markers developed for a distant relative (*Ipomopsis aggregata*) for cross-species applicability in *A. penstemonoides*. Four microsatellites showed amplification of targeted loci and indicate genetic conservatism within the Loeselieae tribe and Polemoniaceae family. Further research is needed to complete an assessment of intraspecific genetic diversity. *A. penstemonoides* is confined to narrowly distributed habitats across a relatively large range, yet retains the ability to occupy a wide array of microhabitats and morphologies within an occupied cliff face. Morphological diversity may be accompanied by genetic or thermotolerance diversity, and may provide a means for adaptation and survival in future climates. Populations are often separated by expanses of unsuitable habitat and appear to have adapted to local rock surface variability. Future research may use *A. penstemonoides* as a model species to test how fragmented populations of a narrowly restricted species respond to a rapidly changing climate.
Acknowledgments

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Dedication

For Kayah Gaydish, who became a mother for so many people and to so many places.

"When a [wo]man dies, people get frightened by the bones, but ones’ essence is not lost. It remains whole and lives in ones’ sons and daughters."

~Popol Vuh

*Aliciella penstemonoides* at the Black Canyon of the Gunnison National Park. Summer 2016. Photograph by Richard Hum
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Foreword

Chapter 1 of this thesis presents an introduction to the ecology of plants living on cliff systems to place Chapters 2 and 3 in context. The research presented in Chapter 2 of this thesis will be submitted to *Conservation Biology* and has been prepared in accordance with the submission guidelines. The third chapter outlines details for further microsatellite marker research and is formatted in the same manner as Chapter 1.

The Painted Wall exemplifies the harsh and difficult to access habitat of *Aliciella penstemonoides* in the Black Canyon of the Gunnison. Summer of 2016. Photograph by Richie Hum.
The author accessing and sampling an occurrence of *Aliciella penstemonoides* in Lake Fork, Colorado during the summer of 2016. Photograph by Gary Walker.
CHAPTER 1: LIFE ON THE ROCKS; THE EFFECTS OF A VERTICAL HABITAT

Abstract

Cliff ecosystems are distributed across the globe and provide unique habitats for many rare or uncommon species that utilize low-competition environments with relatively few natural disturbances. The inherent challenges of accessing cliff ecosystems have historically restricted the investigation of biotic communities occupying such vertical habitats. Recent advancements in climbing equipment and techniques provide a platform for researchers to now discover previously undescribed cliff-dwelling specialists in habitats adjacent to thoroughly catalogued landscapes. Obligatory chasmophytes often occupy narrow distributions and often include endemic species that increase regional measurements of biodiversity. The insular, fragmented habitats of cliff ecosystems present an excellent model system in which to study the consequences of climate change on isolated populations that occupy such unique ecosystems.
Challenges and advantages of chasmophytic habits

Species that dominate the horizontal landscape surrounding cliff systems are often incapable of tolerating the extreme conditions that persist on cliff face habitats and allow cliff-dwelling specialist species to persist in these low-competition environments. Obligatory chasmophytic species are exclusively cliff-dwelling specialists with adaptations that allow the species to withstand extreme edapho-climatic conditions imposed by a vertical habitat (Snogerup 1971; Antonsson 2012). The harsh conditions on cliff faces likely dominate selection criteria and result in habitat specializations that lead to rarity and subsequent development of poor dispersal abilities (Gaston 1995; Colas et al. 1997; Rajakaruna et al. 2014). As a species becomes specialized to a unique habitat, regional distributions become constrained by habitat availability/connectivity, local extinctions and colonization dynamics (Holt & Keitt 2000). By adapting to harsh conditions instead of dispersal ability, chasmophytic species may be at risk of declining population and range sizes under future climate change.

Gravity consistently induces rock fall disturbance regimes and likely drives adaptation in cliff-dwelling organisms. Specialized root adaptations, reminiscent of shallow soil endemics, anchor chasmophytic plants into the substrate and provide access to soil nutrients and water sources tucked deep within tiny cracks and crevices of the rock face (Poot & Lamers 2008). Old growth forests composed of species such as Thuja occidentalis L. and Juniperus phoenicea L., can be found on vertical cliff faces and display gnarled and twisted trunks that can grow in inverted directions to withstand the force of gravity and avoid hazardous rock fall (Walker 1987; Larson et al. 2000; Mathaux et al. 2016). These ancient forests have been protected from anthropogenic disturbance such as fire, grazing,
or land development (Larson et al. 2000). Historically, humans have used cliff systems to find shelter from extreme weather events and as a place to harvest food from cliff-dwelling species (e.g., honey, bird eggs, *Lactuca* spp., *Allium* spp.; Larson et al. 2000). The aesthetic nature of cliffs provide a centerpiece for many National Parks and protected lands and are threatened by mining, quarrying, and river inundation from damming (Larson et al. 2000).

Rock climbing is the main anthropogenic disturbance threatening otherwise undisturbed vertical habitats (Larson et al. 2000, Holzschuh 2016, Boggess et al. 2017). Over the past 20 years, the number of rock climbers in the USA alone has grown to reach over 10 million individuals and has resulted in greater use of established routes, in addition to a greater number of individuals developing first ascents (previously unclimbed routes; Cordell 2012). A review by Holzschuh (2016) assesses the threats of rock climbing to cliff biodiversity and shows that evidence is inconclusive as species response to rock climbing is taxa specific and few studies contain proper controls sites. A number of reports indicate that increased rock climbing activities have a negative impact on cliff face vegetation (Nuzzo 1995; Camp & Knight 1998; Larson et al. 2000; McMillan & Larson 2002; Rusterholz et al. 2004; Clark & Hessel 2015). However, a number of other studies show that climbing has no effect on cliff face vegetation (Nuzzo 1996; Kuntz & Larson 2006; Baur et al. 2007; Adams & Zaniewski 2012; Boggess et al. 2017). Most other large mammal species are unlikely to affect cliff vegetation. However, some goat species (e.g., *Capra ibex* L., *Oreamnos americanus* de Blainville) are excellent climbers and find a release from predation on cliff systems. Small rodent species are commonly observed in improbable locations on cliff systems and likely sustain themselves on chasmophytic herbs (personal observation). Avian species use cliffs to nest and roost in predator-free locations and use
the consistent wind patterns for flight. Avian guano on cliff faces provide a nutrient input that supports nitrophilous lichens (e.g., *Caloplaca* and *Xanthoria* spp.) and demonstrates that cliff ecosystems are maintained by processes that are disparate from the surrounding horizontal landscape (Langevin 2015).

Cliff systems present an abrupt change to the landscape and result in extreme exposures to intense light and high winds that may drive niche adaptations. Light intensity and insolation may be greater for exposed equatorial-facing cliffs than for adjacent horizontal landscapes. Alternatively, poleward-facing cliffs likely receive lower levels of solar radiation when compared to equatorial facing cliffs and horizontal landscapes, and present a dramatically different habitat supporting shade resistant complexes (Nevo 1995). Photooxidative stress occurs in prolonged, high light environments and may disrupt metabolism if antioxidative defenses are insufficient (Foyer et al. 1994). In alpine habitats, adaptations to reduce oxidative damages result in species with accumulated heat shock proteins and large ranges of thermotolerance abilities (Streb et al. 1997a; Streb et al. 1997b; Wang et al. 2004; Kotak et al. 2007). Cracks, crevices, ledges, and overhangs within cliff systems support diverse microhabitats that can shelter an individual from direct exposure and provide a buffer from extreme temperatures. The thermal conductive ability of cliff walls may protect chasmophytic species from cold temperatures during winter months, yet may severely increase temperatures during the summer months. Similarly, insects may be attracted to the warm surface during summer nights (personal observation). The thermal conductivity gradients and abrupt change in topography may provide a near-constant source of wind (Larson et al. 2000). Seeds without adaptations to adhere to the cliff face are quickly removed by wind, as are small soil particles and exposed
limbs or stalks. Prolonged wind, periodic rock fall, and drought-induced damages cause partial cambial mortality and sectoriality in chasmophytic trees, and result in increased lignification and stunted growth (Mathaux et al. 2016). High wind events can shear branches or trunks and promote krumholtz growth forms. Reduced growth forms render smaller targets for falling rocks and increase the possibility for long lifespans (Larson et al. 2000, Sanguin et al. 2016).

Large structural heterogeneities scattered throughout a cliff face (e.g., dihedrals, arêtes, overhangs) provide a buffer from intense light and wind exposure, and play an important role in seed dispersal, distribution, local adaptation, and microclimate diversity (Opedal et al. 2015). The abundance and size of heterogeneities in the rock face affect the density of chasmophytic populations in addition to the amount of moisture released from the cracks and crevices of the rock into the environment (Larson et al. 2000). Cliff faces may appear as dry environments, however the matric potential of rocks holds water molecules at a lesser tension than that of soil particles and allows plants to take up water more readily than when in surrounding soils (Larson et al. 2000). Wet cliff sites will generally be dominated by vascular plants while lichens dominate dry cliff sites (Aho 2014). Individuals located within a crevice may have access to readily available moisture and are sheltered from high winds that can evaporate moisture, displace soil, dislodge roots, and sheer off stalks. Individuals that grow in sheltered microhabitats are capable of producing larger leaves when compared to exposed individuals, and use the larger leaves in the low-light environment to increase photosynthesis (Carmo-Silva et al. 2015; Opedal et al. 2015). Varying levels of light or wind exposure may allow for phenotypic plasticity, or genetic diversity, that select for individuals adapted to varying thermotolerances and as a
result, increase the diversity of microhabitats occupied by the species (Callaway et al. 2003).

**Climate change and the distribution of montane species**

Darwin suggested that abiotic mechanisms govern a species range in abiotically stressful habitats, while their range in more benign habitats will be governed by species interactions (Louthan et al. 2015). Understanding the ecological and evolutionary mechanisms that govern the extent of a species range remain key questions regarding the spatio-temporal dynamics of populations (Sutherland et al. 2013). The fitness of an individual plant may be governed by complex interactions between abiotic constraints (e.g., spatial variation, habitat availability, extreme climatic events) and habitat specializations—establishing a theoretical niche. Biotic factors play an important role in defining the realized niche via colonization dynamics, natural enemies, predation, and inter- and intraspecific interactions (Holt & Keitt 2000; Gaston 2009; Sexton et al. 2009).

While range limitations are temporally flexible and unique to specific taxa, all plant species have individuals that occupy sites at the range margin where adaptations—or the failure to adapt—to a novel environment result in an expansion or contraction to the current distribution of the species (Grubb 1977; Sexton et al. 2009).

Global temperatures are projected to increase by at least 2°C by 2050, as will the frequency and severity of extreme climatic events such as droughts, heat waves, or early winter warming (Easterling et al. 2013; IPCC 2014). Plants will respond to such changes by either 1) migrating to track suitable habitat, 2) adapting to the novel environment within currently occupied sites, or 3) becoming locally extirpated which may amount to global extinctions (Pettorelli 2012). Habitat specialists occupying regionally restricted habitats...
are unlikely to migrate and must therefore rely upon adaptive abilities to withstand future climate scenarios (Krause et al. 2015). Extreme warming events such as heat waves and prolonged drought have a negative effect on the physiological functioning of most plants and may result in retardation of growth and development, causing eventual mortality (Kotak et al. 2007). Warm-adapted species accumulate heat shock proteins that could protect the plant during heat waves, which may extirpate cold-adapted species under the same event (Wang et al. 2004; Kotak et al. 2007; Nevo 2012; Orsenigo et al. 2014). Intraspecific variation in thermotolerance capabilities may exist in species that occupy a variety of microniches, fragmented habitats, or occur along environmental clines (Pratt & Mooney 2013). Under extreme climatic events, individuals with an inadequate level of fitness are removed from the gene pool and allow individuals capable of enduring extremes to reproduce, thereby inducing selection for phenotypes that are compatible with the surrounding environment. Heat waves and other extreme climatic events are more detrimental to individuals at younger developmental stages when compared to mature adult plants (Orsenigo et al. 2014). The demographic structure of a population may therefore be negatively affected and be at risk of genetic bottlenecking by more frequent stochastic warming events (Orsenigo et al. 2014).

Mountain ecosystems provide a heterogeneous landscape with diverse microclimates and high rates of niche evolution and are important biological hotspots (Myers et al. 2000). Tolerances to frost and low levels of precipitation are important factors governing the potential range of many species (Easterling et al. 2000). For many mountain plants, speciation events and adaptive traits have been driven by cold climatic events, glacial recessions, and interglacial periods of warming (Davis & Shaw 2001; Orsenigo et al.
Future stochastic warming events will likely result in the reduction of cold adapted species (Orsengio et al. 2014). Species occupying the relatively hot and arid environment of a south-facing slope are expected to undergo less environmental stress during anticipated global climate change when compared to the relatively cool and moist north-facing slopes (Nevo 2012).

Opposing pole-ward and equatorial facing slopes present divergent evolutionary-ecological systems, and may be considered an “evolution canyon” model system to study the effects of global warming at a microscale level (not to be confused with Sewall Wrights’ fitness landscape; Nevo 2012). North-facing slopes (in the Northern Hemisphere) receive considerably lower levels of insolation when compared to opposing south-facing slopes, and support shade tolerant adaptive complexes (Nevo 2012). Perennial species are more likely to be found on north-facing slopes as abiotic forces select for plants with major adaptations in response to light stress and photosynthesis (Nevo 2012). The comparatively high light intensity of the south-facing slopes selects for species with adaptations to solar radiation, heat, and drought stress (Nevo 2012). A decrease in overall plant cover is to be expected on the high stress-south-facing slopes and will support taxa with increased thermotolerance capacities (Nevo 2012). In comparison, the north-facing slope may have greater plant cover than south-facing slopes, yet stochastic heat waves may have detrimental effects on north-facing community/population composition due to lower thermotolerance capabilities (Nevo 2011; Orsengio et al. 2014). During the winter months, high light and low temperatures may be experienced on south-facing slopes, and may lead to photoinhibition when low temperatures slow the enzymatic processes of the Calvin Cycle (Warren & Robert 2010). A species at the margin of their adaptive zone may be found
on the north-facing slope, but not on the opposing south-facing slope because of the severe stresses that prevent beneficial functioning of phenotype-environment interactions (Nevo 2012; Hermant et al. 2013).

The thermotolerance capacity of a species is an important characteristic to include in the assessment of a species reaction to future climate change. During extreme warming events such as heat waves or droughts, individuals of a population that lack an accumulation of heat shock proteins experience higher rates of mortality, and result in a natural selection process by which the population then becomes more suited to extreme heat events (Parmesan 2006). While tolerances to heat are largely conserved across lineages, the ability for a species to evolve further physiological tolerances to increased temperatures are unlikely, given that the upper thermal limits of the species niche are already occupied (Araújo et al. 2013). Such species whose phenology limits distribution through upper thermal tolerances are not predicted to adapt to further warming (Araújo et al. 2013).

Indirect effects to plant fitness may result from increased warming. In the Western U.S., hybridization between Ipomopsis aggregata and I. tenuituba is the result of overlapping altitudinal ranges, however current warming events are driving hawkmoths to forage for pollen at night when temperatures are cooler and the white flowers of I. tenuituba are more prominent (Aldridge & Campbell 2007). In turn, hummingbirds appear to preferentially select I. aggregata flowers, which subsequently isolates gene flow as the nectar reserves have not been depleted by nighttime foragers. Furthermore, the effect of early winter warming and subsequently earlier flowering periods is expected to disrupt plant-pollinator interactions (Memmott et al. 2007).
**Aliciella penstemonoides**

Members of the Polemoniaceae (Phlox) family have been used as model species for evolutionary investigations into plant speciation (Grant 1966; Wolf et al. 1991; Johnson et al. 2008; Porter et al. 2010). *Aliciella penstemonoides* (Black Canyon gilia) is a Vulnerable plant (Global Status of G3, Colorado State Status of S3; NatureServe 2012), endemic to cliff habitats of the Upper Gunnison river basin, East-Taylor watershed, Rio Grande headwaters, and Uncompahgre river of Colorado (Peterson 1981; Grey 1982; Rondeau 1999; CNHP 2002; Johnston 2002; Beatty et al. 2004). The namesake Black Canyon of the Gunnison (BLCA) is the center of distribution for this species and is dominated by sheer vertical cliff walls that can stretch for over 600 vertical meters above the powerful Gunnison River (Fig. 1.1). As an obligate chasmophyte, populations of *A. penstemonoides* are found exclusively along the vast expanses of vertical rock faces that is accessible only to those using modern climbing equipment and techniques.

Originally described in 1893 near Cimarron, Colorado, as *Gilia pentstemomoides* by M.E. Jones, the Black Canyon gilia has undergone a series of taxonomic revisions. A misprint in an early publication produced the now common scientific epithet of “*penstemonoides*” (Beatty et al. 2004). The misnomer resulted in a common name, the beardtongue gilia, to which the plant bears little resemblance and is a cause of great confusion to those who have not interacted with the species. Molecular and phylogenetic analyses of the Polemoniaceae family recognize a polyphyletic *Gilia* genus and subsequent re-circumscription place the Black Canyon gilia into the Giliandra section of the antiquated genus, *Aliciella* (Porter 1998; Porter & Johnson 2000). Weber and Wittmann (2001) suggest using *Aliciella penstemonoides* under the revised treatment by Porter (1998).
While the principle of priority would consider *A. pentstemonoides* to be the correct nomenclature, its use is causing inconsistencies in herbaria collection databases (e.g., SEINet, Rocky Mountain Herbarium). The use of *Aliciella penstemonoides* (M.E. Jones) J.M. Porter is therefore chosen in the current research as *Gilia penstemonoides* is considered an orthographic variant by the Integrated Taxonomic Information System (ITIS).

The lack of detailed abundance records is unsurprising given the vast extent of largely inaccessible topography occupied by *A. penstemonoides*. At such an immense scale, the ability to effectively access and survey habitat for *A. penstemonoides* hinges on the safety and ability to use technical rope access. Since its original discovery, ca. 28 known occurrences of *A. penstemonoides* have been recorded from Gunnison, Hinsdale, Mineral, Montrose, and Ouray Counties. The disjunct populations of *A. penstemonoides* are intermittently dispersed along cliff systems that border water drainages and appear to have crossed the continental divide between Cathedral and Creede in southern-central Colorado (Fig. 1.2). While occurrences may be distributed across a large geographic extent the selective chasmophytic nature of this species allows for the plant to be defined as an edaphic endemic (Ferreira & Boldrini 2011). It is likely that occurrences remain undiscovered and abundance may be greater than what is currently described. However, the inaccessible nature of the topography has left nine of the 22 reports entirely deficient of abundance data, while 15 occurrences have not been observed since 1995 or earlier—including two populations presumably extirpated by the flooding of the Blue Mesa Reservoir in 1978 (Peterson 1981; CNHP 2002; Beatty et al. 2004). The vertical topography occupied by *A. penstemonoides* hinders disturbances that commonly threaten other rare Colorado endemics, while additionally deterring researchers from easily accessing
occupied sites (O’Kane 1988; Johnston 2002; Beatty et al. 2004). As a consequence, little is known about species abundance, ecological requirements, or genetic variation (Porter 1998; Porter & Johnson 2000).

With an affinity for vertical cliff faces, *A. penstemonoides* individuals inhabit narrow crevices with sufficient depth for root anchorage (Beatty et al. 2004). Little soil accumulation occurs in small crevices, making it difficult to sample and analyze. However, Grey (1982) was able to conclude that soils supporting *A. penstemonoides* populations are low in carbonate, high in potassium and iron (as compared to other Colorado soils), mostly of sandy texture but with high portions of silts and clays, and possess varied pH levels (6.1-7.5) and low percent organic matter (4.2-7.8). Four main soil associations are documented by Grey (1982)—Posant-Woodhall-stony rock land, Shule-Youman-Passar, Parlin-Lucky Hopkins, and Torriorhents-rock outcrop—all of which are classified as stony rock land or rock outcrop series (Beatty et al. 2004). *A. penstemonoides* is thought to prefer damp, rocky soils and does not appear to grow in deep pockets of soil or in open areas (Beatty et al. 2004). Distribution along cliff habitats varies, but likely depends upon surface heterogeneity and may increase in abundance with increasing proximity to river systems (Beatty et al. 2004).

As an obligate outcrosser, *A. penstemonoides* is pollinated by Bombus, Megachilidae, and Halictidae, yet seed dispersal mechanisms are not fully understood (Grey 1982; Beatty et al. 2004). Individuals reportedly produce 22 to 37 capsules and approximately 7 to 12 mucilaginous seeds per capsule, which are hypothesized to be small and light enough to be carried by the wind, but which can adhere to water and soils running down cliff faces (Grey 1982). A tetrazolium viability test on 50 seeds indicated that only 8% of seeds were
strongly viable following cold stratification, yet germination rates appeared to be the
greatest after an eight-month cold stratification (17-24%; Grey 1982). This same study
estimated the reproductive capability of *A. penstemonoides* to be ca. 50% due to low seed
production and poor germination success. A perennial life history, low rate of seed
propagation, and the ability to withstand harsh, unproductive conditions, define *A.
penstemonoides* as a stress-tolerant species (Barbour et al. 1985, Beatty et al. 2004).

The Giliandra section is the basal lineage of the *Aliciella* subgenera and shares
common ancestry with species that use low competition habitats. The extremely rare and
closely related *A. sedifolia* (Brandegee) J.M. Porter is found exclusively on the talus slopes at
or above tree line on Half Peak in Hinsdale County and is approximately 47 kilometers from
the nearest record of *A. penstemonoides*. The abiotic controlling processes that govern
adaptation in alpine environments are similar to those of cliff ecosystems (Larson et al.
2000). The widespread *A. pinnatifida* (Nutt. ex A. Gray) J.M. Porter appears to have
speciated at the same time as *A. sedifolia* and *A. penstemonoides* (Porter 1998). Southern
portions of *A. penstemonoides* range are thought be hybridizing with *A. pinnatifida*, while
strong morphological similarities are apparent with *A. mcvickerae*. The extreme
environmental conditions occupied by members of the *Aliciella* subgenera may incur
morphologically static adaptations as physical characteristics converge on forms that are
best suited for the harsh conditions (Bickford et al. 2006).
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The author after locating *Aliciella penstemonoides* during a rappel in the Black Canyon of the Gunnison National Park during the summer of 2016. Photograph by Kate Lis.
Figure 1.1: The Painted Wall (685 meters) in the Black Canyon of the Gunnison National Park compared to (1) Great Pyramids (137 meters), (2) Washington Monument (169.2 meters), (3) Eiffel Tower (324 meters), (4) Empire State Building (381 meters), (5) Willis Tower (442 meters), (6) Tokyo Sky Tree (634 meters), and (7) Burj Khalifa (828 meters). Courtesy of the Black Canyon of the Gunnison National Park Service.
Figure 1.2: The 28 known occurrence locations of *A. penstemonoides* throughout Colorado.
CHAPTER 2: POPULATION INVENTORY, THREAT ASSESSMENT, AND ANTICIPATED EFFECTS OF GLOBAL CLIMATE CHANGE ON AN ENDEMIC, CLIFF HABITAT SPECIALIST, ALICIella PENSTEMONOIDes (POLEMONIACEAE)

For submission to Conservation Biology

Abstract

Cliff ecosystems provide habitat specialist species with a low-competition environment where they experience relatively few disturbance regimes. Aliciella penstemonoides (M.E. Jones) J.M. Porter exclusively occupies vertical cliff walls that line rivers and streams of the Upper Gunnison River Basin and Headwaters of the Rio Grande in southwest-central Colorado. Due to the difficulties in accessing cliff habitats, few studies have focused on A. penstemonoides, and little is known about overall abundance, ecological requirements, genetic diversity, or susceptibility to climate change. Fieldwork in the summer of 2016 concluded that A. penstemonoides is a regionally rare, yet locally abundant species that incurs minimal disturbances. To estimate the threat climate change imposes on A. penstemonoides, I constructed a species distribution model (SDM) using occurrence locations from the summer of 2016 along with current climate models, and three projected climate scenarios for the year 2050. All projected models indicate a major loss of suitable habitat with no expectations for range expansions. While all models indicate that climate change may impose a severe threat to the distribution of A. penstemonoides, data representing adaptive abilities, migratory possibilities, and extinction rates are lacking. Nevertheless, the strong model indicates that A. penstemonoides will become threatened by climate change and must either adapt, tolerate, or migrate to survive.
Introduction

The ongoing biodiversity crisis necessitates the need for the assessment of rare and endemic taxa so that we may recognize how global climate change influences species distributions and understand when local extirpations may lead to global extinctions (Obregón et al. 2016). Restricted taxa face unique conservation and management challenges due, in part, to the difficulties associated with measuring the extent of occupied habitat, and anticipating range shifts under future climates (de Castro Pena et al. 2014). Edaphic endemics, or rare plants confined to resource-limited soils, are of particular concern as suitable habitats are often highly fragmented, and dispersal mechanisms or specialized adaptations limit distribution and range expansion (Ferreira & Boldrini 2011). Cliff ecosystems are a geological landscape anomaly in western Coloradan landscapes and provide naturally fragmented habitats with definitive biological community boundaries and release from interspecific competition and anthropogenic disturbance (Larson et al. 2000; Kelso et al. 2001; Antonsson 2012). Cliff habitats present high stress environments as a result of verticality, and create strong environment-species interactions that result in regionally-restricted insular habitats for cliff-dwelling exclusive plants, known as obligate chasmophytes (Runemark 1971; Snogerup 1971; Larson et al. 2000). These species provide an excellent model for studying the effects of climate change and isolation on a species with definitive habitat boundaries, regional restrictions, and minimal anthropogenic disturbances (Antonsson 2012).

Novel methods for depicting the spatial extent of endemic taxa are becoming increasingly important for use by land managers who want to develop management plans that protect species under projected climate change. Species distribution models (SDM)
incorporate occurrence records of individual taxa along with environmental predictors (e.g., geological, topographical, and climate) on a geographic information system (GIS) platform, to estimate the realized niche of a species and can be used to locate new occurrences (Williams et al. 2009; Araújo & Peterson 2012). Models may incorporate projected climate scenarios to estimate the extinction risk for a species under continued anthropogenic-induced climate change (Araújo & Peterson 2012). Areas of potential distribution under future climate scenarios are inferred from present day locations (Araújo & Peterson 2012). Consequently, variables that prohibit a species from occupying apparently suitable habitat (e.g., dispersal limitations, competition, historical factors, or unknown abiotic variables) are difficult to model and can result in an overprediction of habitat deemed suitable by the model (McCune 2016). Models aimed at assessing the risk of climate change on species distributions should therefore be treated with caution, but are important and useful tools for establishing conservation and management plans for a species that may incur future range shifts (Hijmans & Graham 2006; Thuiller et al. 2008; Merow et al. 2013). The following study assesses the possible implications of climate change on the distribution of suitable habitat for a specialist species that receives minimal direct anthropogenic disturbance by growing exclusively on cliff faces.

_Aliciella penstemonoides_ (M.E. Jones) J.M. Porter (Polemoniaceae; also known as the Black Canyon Gilia and _Gilia penstemonoides_) is a Vulnerable plant (Global Status of G3, Colorado State Status of S3; NatureServe 2012), endemic to vertical cliff habitats that line creeks, rivers, and reservoirs (Peterson 1981; Grey 1982; Rondeau 1999; CNHP 2002; Johnston 2002; Beatty et al. 2004). The namesake Black Canyon of the Gunnison is the estimated center of distribution for this species and is epitomized by sheer vertical cliff
walls that can stretch for over 600 vertical meters above the Gunnison River. As an obligate chasmophyte, *A. penstemonoides* is found exclusively along vertical rock faces that is only accessible with the use of modern climbing equipment and techniques.

Detailed abundance records are lacking given the vast extent of largely inaccessible topography occupied by *A. penstemonoides*. Since its original discovery in 1893, approximately 28 known occurrences of *A. penstemonoides* have been recorded in Gunnison, Hinsdale, Mineral, Montrose, and Ouray Counties. Of the 28 occurrences, nine are entirely deficient of abundance data, while 15 occurrences have not been observed since 1995 or earlier—including two populations presumably extirpated by the flooding of the Blue Mesa Reservoir in 1978 (Peterson, 1981; CNHP 2002; Beatty et al., 2004). Populations of *A. penstemonoides* follow cliff systems that intermittently border water drainages to the south and east of the Gunnison River in Gunnison and Montrose County, and cross the continental divide between Cathedral (Hinsdale County) and Creede (Mineral County) of south-central Colorado. While occurrences may be distributed across a large geographic region the chasmophytic nature of the species allows for *A. penstemonoides* to be described as an edaphic endemic (Ferreira & Boldrini 2011). It is possible that populations have yet to be discovered. The vertical topography occupied by *A. penstemonoides* limits disturbances that commonly threaten other rare Colorado endemics, while additionally deterring researchers from accessing large portions of apparently suitable habitat (O’Kane 1988; Johnston 2002; Beatty et al. 2004). As a consequence, little is known about this species abundance, ecological requirements, or genetic variation (Porter 1998; Beatty et al. 2004).
The current study has three main objectives: 1) to locate and describe the current distribution of *Aliciella penstemonoides* and possible hybrids, 2) to assess threats impending individual mortality, and 3) to generate a species distribution model of *Aliciella penstemonoides* with a series of 2050 climate predictions.

**Methods**

*Study species*

Occurrences of *A. penstemonoides* are documented on both sides the Western Continental Divide with intermediate morphological characteristics between *A. penstemonoides* and *A. pinnatifida* (Nutt. Ex A. Gray) J.M. Porter occurring south of the Gunnison River. *Aliciella penstemonoides* is distinguished from *A. pinnatifida* by a perennial growth form and chasmophytic habit. *Aliciella pinnatifida* is an annual plant that can be found in gravelly and barren soils in the foothills to subalpine habitats of Colorado, Kansas, Nebraska, New Mexico, Utah, and Wyoming. To maximize finding the potential range of *A. penstemonoides* considered in this study, I included all occurrences of *A. penstemonoides*, or possible hybrids, located on cliff faces or those individuals that retained a previous years stalk within the expected range.

*Study area*

Two million years ago, the Gunnison River began rapidly downcutting into the Precambrian core of the exhumed Gunnison uplift at a rate of 30 centimeters per millennium (Hansen 1965). The result is a 77-kilometer long chasm in the Colorado Plateau region with sheer vertical cliff walls descending over 600 m on either side of the Gunnison River (Hansen 1965). Within the Black Canyon of the Gunnison National Park (BLCA),
habitat for *A. penstemonoides* is restricted to cliff faces and vertical portions of large boulders at or below the rim of the canyon in areas requiring wilderness permits. Up river of the BLCA, the Gunnison River extends into the Southern Rocky Mountain region where habitat of *A. penstemonoides* occurs on riparian cliff faces owned and managed by the Curecanti National Recreation Area (CURE). Populations extend through intermittent cliff systems of southern drainages, and continue into the northeast corner of Hinsdale County along Cebolla Creek where property is owned and managed by United States Forest Service Rocky Mountain (USFS), Bureau of Land Management (BLM), and a variety of private landowners. The Continental Divide bisects the distribution of *A. penstemonoides* and occurrences are reported in Mineral County. The East-Taylor watershed in Gunnison County houses a disjunct population and specimens have been collected from single occurrences in the Uncompahgre Gorge of Ouray County, and the West Elk Mountains north of the Blue Mesa Reservoir.

**Occurrence data**

In the BLCA and CURE, I accessed cliff habitats supporting *A. penstemonoides* by rappelling from the rim of the canyon. In locations outside of the BLCA and CURE, identification of *A. penstemonoides* took place at the base of the cliff systems with the assistance of a spotting scope and binoculars, and I used climbing equipment and techniques when needed. Technical access to vertical terrain met or exceeded standards of the American Mountain Guides Association. I used a spotting scope and binoculars to survey cliff walls across narrow chasms, large rivers, or nearby rock walls in accessible terrain. Following positive field identification of *A. penstemonoides*, I recorded locations using a Garmin Oregon 450 GPS device. GPS device accuracy contained errors up to nine
meters because of large cliff systems and low satellite reception. I plotted coordinates in ArcGIS (ESRI, Redlands, CA) and corrected for errors during visual analysis of point locations in ArcMap 10.3.1 (ESRI, Redlands, CA). I collected rock samples for later identification by Anthony Love of the Appalachian State University Department of Geological and Environmental Sciences.

*Data loggers*

I placed Onset HOBO® UA-002 Pendant Temperature Light Data Loggers (Onset Computer Corporation, Bourne, MA) near healthy individuals within seven populations of *A. penstemonoides*. I attached data loggers to removable climbing equipment and fixed in a horizontal position using zip ties. Data loggers measured average temperature (°C) and light intensity (lum/ft²) at hourly intervals and simultaneously collected data between July 4th and July 28th of 2016. Temperature recordings are likely inflated due to greenhouse effects within the waterproof casing and were not used in the construction of the SDM (Appendix 1; da Cunha 2015). The gathered data are therefore unreliable and are not discussed further. I cleanly removed all equipment from the rock faces following the conclusion of research.

*Distance sampling / Threat assessment*

I pooled verified populations of *A. penstemonoides* to be assessed for density and threats. I selected three populations west of the Continental Divide and two east of the Continental Divide following an assessment of accessibility and safety. Within each population, I established five line transects in terrain that appeared suitable to *A. penstemonoides* and could be safely accessed. I employed distance sampling line transect
methods and followed assumptions proposed by Buckland et al. (2001). A rappel rope allowed safe access to vertical terrain and simultaneously served as a line transect. I measured the perpendicular distance from the rappel line to each observed individual within 1.5 meters of either side of the rappel line. At each encountered individual, I recorded the perceived threat to individual mortality. I truncated measurements to 1.5 meters as the detection rate experienced a noticeable decrease beyond this distance. Rock climbing was recorded to be a threat when the plant occupied a crack or crevice that could be used as a climbing hold on an established climbing route. Herbivory was recorded when the flowering stalk was sheered from the basal rosette without noticeable damage to remainder of plant. When *A. penstemonoides* shared rooting space in a crack or crevice with another plant species, I recorded a threat to interspecific competition. Chlorosis was recorded on plants that lacked pigmentation. I recorded a threat of rock fall when damage to flowering stalks and basal rosettes was observed at a site that did not support an obvious climbing route. When plants possessed a desiccated stalk or basal rosette, I recorded a threat to dehydrated. While multiple threats could be observed for a single individual, the primary threat that appeared to place the plant at the greatest risk of mortality was recorded. Transect length was dependent upon cliff height, except in the BLCA or CURE where a 60 meter rope was the limiting agent. I analyzed data collected from distance sampling with the R package ‘Distance’ (Miller 2016).

*Environmental data and projected climate data*

To delimit the ArcMap 10.3.1 (ESRI, Redlands, CA) workspace for the species distribution model, I used level five Watershed Boundary Datasets from the National Hydrography Dataset by the U.S. Geological Survey (USGS) to create a layer of watersheds
containing a known occurrence of *A. penstemonoides*, and all neighboring watersheds. A 1/3 arc-second digital elevation model (DEM) was mosaicked from data obtained from the USGS National Elevation Dataset, and clipped to cover a rectangular landscape encompassing the extent of watersheds in the previous layer. The rectangular boundary encompassed approximately 4,200,000 hectares (ha) of southwest-central Colorado. The workspace in ArcMap was set to match the same geographic extent, cell size, and spatial reference as the DEM layer used to delimit the model boundary. To account for large cliff systems that may be undetected by the slope layer I derived aspect and slope from the DEM layer and calculated topographic ruggedness index (TRI) within a 3x3 neighborhood focal statistic with the following equation:

$$\text{Eq. 1 TRI} = \sqrt{|G^2 - H^2|}$$

where $G$ is the maximum value in a 3x3 neighborhood and $H$ is the minimum value in a 3x3 neighborhood.

Geologic substrates are strong predictors of endemic plant distributions in the Colorado Plateau region (Krause 2010, 2012). I acquired USGS geology maps and created a categorical raster layer of the primary rock type. Soil information was omitted from the model as spatial resolution would unlikely capture soils documented by Grey (1982).

The Climate Adaptation Conservation Planning Database for Western North America (CACPD) project provides current and projected climate variables at one-kilometer resolution (Hamann et al. 2013). I obtained 24 bioclimatic variables calculated from yearly averages between 1961 and 1990 (30 year averages account for yearly oscillations), and projected climate models for the year 2050 (Appendix 2; Hamann et al. 2013). Data from the CACPD represent socio-economic driven SRES A1B emission scenarios, defined by the
Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report (AR4) as a rapidly-growing economy fueled by a balance of fossil and non-fossil fuels (IPCC 2007). CSIRO_MK3_0 (Australia, 1.87°x1.87°) represents the cool/moist projection with a 1.9°C rise in temperature and 1% increase in precipitation. UKMO_HADCM3 (UK, 1.87°x1.24°) is the median projected scenario with a 3.4°C rise in temperature and a 3% increases in precipitation. IPSL_CM4 (France, 3.75° x 2.50°) represents the warm/dry scenario for 2050 with a 4.1°C rise in temperature and 2% drop in precipitation.

MaxEnt model

The ability to minimize the relative entropy between two probability densities allows maximum entropy niche models (MaxEnt) to outperform other niche modeling programs—particularly with small population datasets (MaxEnt version 3.4.1; Phillips et al. 2017). I enabled random seed to ensure that the same set of pseudorandom numbers generated per run was not replicated, in addition to setting aside 25% of the occurrence locations to test the accuracy of the model over the 15 subsample replicate run (Young et al. 2011). To conserve time and computer processing, I de-selected the “Write clamp grid when projecting” box. I set the maximum iterations to 5000 and de-selected the “Write output grids” box. I kept the remaining settings at default.

Species distribution modeling evaluation

To quantify different attributes of the predictive strength of the model, MaxEnt software evaluates performance via two methods: 1) a threshold-dependent binomial test based on omission and predicted area, and 2) a threshold-independent receiver operating characteristic (ROC) analysis, which characterizes the performance of a model at all
possible thresholds by a single number, the area under the curve (AUC; Phillips et al. 2006; Elith & Leathwick 2009). The average omission and predicted area binomial test uses the extrinsic omission rate (i.e., the fraction of test points that fall into pixels not predicted suitable) and the proportional predicted area (commission rate; Phillips et al. 2006). The relationship between sensitivity (i.e., percentage of presences correctly predicted) and specificity (1 minus the percentage of absences correctly predicted) is represented by the ROC curve (Krause et al. 2015). The ability of the model to correctly classify a species as present or absent is measured by the AUC using presence versus random background points (Phillips et al. 2006). AUC values of 0.9-1 are considered models with excellent accuracy (Araújo & Guisan 2006).

MaxEnt software additionally supplies an analysis of variable contributions table to determine the predictive strength of independent variables. The increase in regularized gain from each iteration of the training algorithm is added or subtracted (if \( \lambda < 0 \)) to the contribution of the corresponding variable to calculate the percent contribution (Phillips et al. 2006). Percent contribution relies upon the algorithm pathway used by the model, while the permutation importance measures the contribution of each variable when randomly permuted throughout the algorithm (Phillips et al. 2006). I employed a jackknife test as a complimentary method to estimate variable importance. MaxEnt software created several jackknife estimate models where each variable was excluded in turn from a model using all remaining variables (without variable) and another model created from each variable in isolation (with only variable; Phillips et al. 2006).

The efficacy of the species distribution model relies upon the ability of the interpreted results to be neither contradicted, conjectural, nor grossly implausible with the
component of the species geographical or environmental range that is being estimated (Araújo & Guisan 2006; Peterson et al. 2011). It is important to establish a target for the modeling exercise and to incorporate data that effectively represent the interpreted results of the model (Araújo & Guisan 2006). Here, we seek to model the changes in the extent of suitable habitat for *A. penstemonoides* under future scenarios of anthropogenic-induced climate change. I therefore chose to use all bioclimatic layers that may identify suitable habitat regardless of correlations that may exist between layers. Consequently, it is not possible to assess the predictive capability of individual layers (Araújo & Guisan 2006).

*Modeled response to climate change*

Using ArcMap 10.3 (ESRI 2011), I converted the output predictions of habitat suitability from continuous to binary (suitable/unsuitable) values using a 10-percentile training presence logistic threshold value from the MaxEnt output. I calculated change in suitable area between current and projected models with binary SDM layers and the following equation:

\[
\text{Eq. 2 } \Delta_{\text{SH}} = \left[ \frac{(A_2 - A_1) \times 100}{A_1} \right]
\]

where \( \Delta_{\text{SH}} \) is the change in suitable habitat, \( A_1 \) is the number of cells suitable under the current climate model, and \( A_2 \) is the number of cells in the future climate model.

To calculate the percent of habitat that is to remain suitable under projected climates, I added the current binary SDM layer to each of the projected binary SDM layers with the raster calculator. Cells with a value of “0” are sites where neither expansion nor contraction occur. Cells with a value of “1” are sites of either expansion or contraction. A value of “2” is a site that remains suitable under the future climate projection. I input the
number of cells with a value of “2” into the overlap variable \( V_2 \) in the following equation, where \( \cap_{SA} \) is the suitable area overlap and \( A_1 \) is the number of cells suitable under the current climate model:

\[
\text{Eq. 3 } \cap_{SA} = \frac{V_2 \times 100}{A_1}
\]

To calculate habitat expansion (habitat that becomes suitable in projected models) I multiplied the output raster from Equation 2 with the projected climate model. The resulting raster contains cells with a value of “1” which indicate cells where habitat expansion occurs under the future climate projections. I divided the number of cells with a value of “1” by the total number of unsuitable cells in the current binary SDM to determine the percentage of cells that become suitable under projected climate. The difference in the number of cells with a value of “0” between the two map algebra outputs is the number of cells experiencing a range contraction.

**Results**

**Occurrence**

Field surveys in 2016 resulted in 2,026 observed individuals of *A. penstemonoides* from 14 populations throughout a variety of land management areas and counties (Table 2.1). I collected 64 non-duplicate GPS locations at sites supporting *A. penstemonoides* between 2130 and 2980 meters (a.s.l.). The anticipated center of distribution is located along the massive cliff walls that line the BLCA and CURE in Montrose and Gunnison Counties, Colorado. Populations extend through intermittent cliff systems of southern drainages, such as Lake Fork and Blue Creek, and continue into the northeast corner of
Hinsdale County along Cebolla Creek where the Continental Divide bisects the remaining disjunct populations of *A. penstemonoides*. Occurrences enter the east side of the divide in Mineral County along Willow Creek and extend into Antelope Park and Bellows Creek. Surveys revealed new localities of *A. penstemonoides* at the northern range limit in the East Taylor watershed, and in the southern range limit in Shallow Creek water district of the Rio Grande. Surveys failed to locate previously documented populations in the Ouray Ice Park in Ouray County, on northern drainages of Snowshoe Mountain in Mineral County, and in the West Elk Mountains north of the Blue Mesa Reservoir. Private property made some sites inaccessible while poor rock quality and hazardous terrain deterred access to other sites. All cliff sites supporting *A. penstemonoides* were found on cliff systems immediately adjacent to riparian habitats.

Habitat supporting *A. penstemonoides* is surrounded by an assortment of ecological systems that are uninhabitable to this species (Fig. 2.1). Taxa that characterize grasslands, sagebrush, piñon-juniper forests, and oak-mixed mountain shrub lands are not found on cliff habitats likely because of the vertical habitat and the lack of specialized adaptations. The habitat occupied by *A. penstemonoides* allows the plant to maintain minimal interactions with competing species. An occurrence in Mineral County contained a cluster of approximately fifteen basal rosettes of *Draba smithii* Gilg ex O.E. Schulz (G2/S2) in habitat that otherwise appeared suitable to *A. penstemonoides*. Rock type varied between populations (Table 2.2) and presented a variety of exposed and sheltered microhabitats. Regardless of the rock type, all substrates supporting *A. penstemonoides* fractured via either joint cleavages or foliation and did not constitute pocketed rock. Distribution of *A.
penstemonoides occurrences are restricted to vertical cliff habitats and surrounded by a variety of Terrestrial Ecological Systems (Fig. 2.2; CNHP 2011).

Density / Threats

Density of A. penstemonoides varied within and among sites, as did threats to individual mortality (Table 2.3 and 2.4). A single 16 meter transect at Lake Fork yielded over 300 individuals of A. penstemonoides while multiple transects of 60 meter on the north rim of BLCA recorded no occurrences. Many individuals appeared to be undisturbed, however individuals were impacted by rock climbing activities at sites with high quality rock (Table 2.4). High quality rock support safe recreational rock climbing activities, and was restricted to populations in the BLCA, Taylor Canyon, Spring Creek, and Lake Fork sites. Rock climbing caused damage to flowering stalks from rope drag and inconsiderate placement of hands or feet. At sites in Hinsdale and Mineral Counties, rock quality was generally poor and consisted of unappealing rock that provided an unsafe site for recreational climbing activities. Interspecific competition was rarely noted when A. penstemonoides appeared immediately adjacent to other taxa occupying the same crack or crevice. I rarely observed flowering individuals to occupy habitat in direct competition with other species, yet the presence of Bromus tectorum L. was commonly noted either competing with A. penstemonoides or occupied neighboring sites that appeared suitable to A. penstemonoides. I noticed signs of chlorosis throughout populations; however, few appeared to be at an imminent risk of mortality. Dehydration was observed in only a few individuals from most sites and an individual in Taylor Canyon supported colorful and mature flowers atop a wilted and desiccated stalk and basal rosette.
**Model output**

The test omission rate and predicted area closely followed the predicted omission rate and contained minimal fluctuations above and below the predicted omission line (Fig. 2.3A). Fifteen replicate runs resulted in an AUC value of 0.986 with a standard deviation of 0.008 and indicated a robust model (Fig. 2.3B). Of the 31 environmental predictors, slope had the highest gain when used in isolation. However, there was a small decrease in gain when slope was omitted suggesting that correlations exist between associated topographic extremes (Table 2.5, Fig. 2.4). Elevation showed the greatest decrease in gain when omitted, suggesting it provides the most useful independent information. Geologic substrate showed the second greatest increase in gain when in isolation and the second greatest decrease when omitted. Of the climate variables, annual temperature made the greatest contribution to the model, however it had little importance when randomly permuted in the training algorithm. Several environmental variables correlate with one another making it problematic to delimit the biological significance of any single variable.

**Modeled response to climate change**

To determine *A. penstemonoides* vulnerability to climate change by the year 2050, I converted the median MaxEnt file outputs into binary layers of suitable versus unsuitable habitat and translated the number of cells into total area in kilometers. The present-day model indicates that 50,976 ha provide suitable habitat for *A. penstemonoides* on a two dimensional surface. By 2050, the CSIRO_MK3.0, UKMO_HADCM3, and IPSL_CM4 scenarios indicate that the habitat currently occupied by *A. penstemonoides* will decrease to 15,505 ha, 13,026 ha, and 312 ha respectively (Fig. 2.5). The future climate models predict no
range expansion (less than 0.002% in all models) by the year 2050 with minimal overlap (2.53%, 0.98%, and 0.01%) between present day and future models (Fig. 2.5 and 2.6).

Discussion

*Aliciella penstemonoides* is an edaphic endemic and obligate chasmophyte that occupies an insular habitat likely due to highly-specialized adaptations and poor-competitive abilities. While it is locally abundant within occupied sites, the distribution of *A. penstemonoides* is narrowly restricted to cliff systems lining rivers and streams of the Upper Gunnison River Basin, East Taylor Watershed, and Headwaters of the Rio Grande. *Aliciella penstemonoides* was observed to possess a deep growing root structure that likely anchors the plant into fracturing joints with deep crevices. Although not measured here, the deep growing root structure may be ineffective in rock outcrops with pocketed faces such as those in the West Elk Mountains. Further research is needed to confirm if such adaptations impose range limits. Morphological variation was large both within and among populations of *A. penstemonoides* (Appendix 3). However, local variation in geologic structure and surface heterogeneities appear to select for unique traits that are locally prevalent.

Individuals of *A. penstemonoides* occupy a variety of microhabitats found across a cliff system and local distributions appear to be governed by the abundance of surface heterogeneities and local adaptations that allow a diverse series of both exposed and sheltered crevices to be occupied. Seeds of *A. penstemonoides* are extremely small (<0.5 mm) and are hypothesized to be distributed by wind and adhere to cliff faces by mucilaginous properties during rain and water runoff (Grey 1982; Beatty et al. 2004). Seeds likely land on horizontal habitats, yet may be incapable of germination and surviving
competition in such sites. Individuals tend to be found following paths of water runoff and are absent from sections of a cliff face below steep headwalls or overhangs. Small growth forms may be a result of the shallow soil environments in small heterogeneities throughout a cliff face where deep growing roots can access nutrient-rich pockets of soil in cracks and crevices that retain moisture longer than the surrounding rock landscape (Poot & Lambers 2008).

*Aliciella penstemonoides*’ stress-tolerant growth form and life-history strategy allow the plant to tolerate natural disturbances such as high winds, drought, and herbivory. The presence of *A. penstemonoides* at sites with heavy anthropogenic disturbances support a theory that this is a disturbance-tolerant species. Anthropogenic disturbances may have begun during the silver mining era near the town of Creede in 1890. However, the presence of *A. penstemonoides* had yet to be described. Construction and inundation of the Blue Mesa Dam began in 1962 and is thought to have extirpated two historical occurrences of *A. penstemonoides* (Grey 1982).

Locations of undiscovered populations of *A. penstemonoides* may be found from ground truth surveys that validate the effectiveness of the model generated using the current climate data. The SDM for the 1961-1990 climate indicates that Cochetopa Canyon in Saguache County supports suitable habitat for *A. penstemonoides*, as do the cliffs bordering Tomichi Creek near Sargents, CO. Sites near Almont in Gunnison County show highly probable locations suitable for *A. penstemonoides*, as do sites along Cebolla Creek where previously documented locations occur. I did not conduct surveys at such sites in 2016. In March of 2017, snow pack, freeze-thaw rock fall, and absence of flowers hindered surveys in Cochetopa Canyon and I did not successfully locate *A. penstemonoides*. Multiple
sites in Mineral County near Creede appear to be suitable habitat for *A. penstemonoides* and overlap previously documented occurrences. Surveys in 2016 near Deep Creek and Marshal Park Campground in Mineral County did not successfully identify individuals of *A. penstemonoides*. Similar predictions for sites north of the Blue Mesa Reservoir in the West Elk Mountains of Gunnison County appear to support suitable habitat, yet surveys in 2016 did not successfully locate *A. penstemonoides* at those sites.

MaxEnt models using projected climate data indicate that the majority of habitat currently occupied by *A. penstemonoides* will likely undergo significant climate change by 2050. Strong models and high AUC values indicate a robust model. Topographic environmental variables made the greatest contribution to training the model, yet no changes in topography had been included in the environmental predictors. Change in the distribution of suitable habitat is therefore likely a result of a changing climate. Under the CSIRO_MK3_0 “cool / moist” scenario, less than three percent of the habitat deemed suitable in the current model is predicted to remain suitable by the year 2050 with essentially no habitat expansion expected to occur. Less area of overprediction is observed in the CSIRO_MK3_0 “cool / moist” projection and may indicate a more accurate model of the extreme climate experienced in cliff ecosystems. Current global temperatures are rapidly approaching the CSIRO_MK3_0 projection; the first three months of 2016 experienced temperatures 1.5°C above that of pre-industrial levels (Climate Central 2016). It is therefore likely that the 2050 climate will exceeded that of the CSIRO_MK3_0 projection. A rapidly growing economy with an increased reliance on fossil fuels suggest that the climatic niche identified by the current day SDM will likely be eliminated from the
landscape by 2050, and will likely be a major driver of adaptation or extirpation for *A. penstemonoides*.

Narrowly distributed taxa facing severe climatic changes, such as *A. penstemonoides*, have three options: 1) physiologically adjust under a new set of environmental conditions, 2) adapt at a local population level and become capable of tolerating the new climate, or 3) become extirpated locally which may amount to global extinction (Pettorelli 2012). Similar studies report analogous results for distribution models of endemic taxa in the Colorado Plateau region and Southern Rocky Mountains (Queiroz et al. 2012; Krause et al. 2015; Still et al. 2015). Edaphic endemic species undergo strong selective pressures regarding species-environment interactions and subsequently lack effective-long distance dispersal mechanisms as the selection for such dispersal is outweighed by selection for survival (Krause et al. 2015). Under the current velocity of climate change, endemic species adapted to extreme environments do not possess the ability to migrate to new habitats in search of suitable habitat (Loarie et al. 2009). *A. penstemonoides* appears to have maintained stable populations as many populations are located near the vicinity of historical records. Evidence supporting the large thermotolerance capabilities of *A. penstemonoides* may indicate that the species could retain the capacity to withstand greater temperature extremes than that accounted for by the modeled response to climate change. It therefore appears that while *A. penstemonoides* may be highly threatened by climate change, it may nonetheless possess the ability to adapt and or tolerate to a changing climate.

Mountain plant communities face extreme environmental conditions with high rates of endemism resulting from high variability in topography and abundance of microclimates. Cliff ecosystems provide refugia for several relict taxa that have been either
outcompeted or otherwise extirpated from the surrounding horizontal vegetation matrix (Runemark 1971; Snogerup 1971). Cliff ecosystems do not support species that typify the surrounding ecological system patches and maintain independent ecological processes (e.g., soil formation, nutrient cycling). Cliff ecosystems maintain independent ecological processes and do not support species that typify the surrounding landscape. Therefore, cliff communities should be considered a unique ecosystem patch. The difficulties in accessing vertical habitats has protected many chasmophytic species from anthropogenic disturbance and has prevented many species from being well documented. By using novel sampling methods, the present research demonstrates that a chasmophytic species may maintain a greater abundance and distribution that what was previously documented. The advances in climbing equipment and techniques are additionally placing species such as *A. penstemonoides* under unfamiliar disturbances. Additional land development such as mining and river damming are undoubtedly deleteriously influencing individual reproductive output, patch connectivity, and source-sink dynamics.

Understanding the vulnerability of a species to climate change can have considerable influence on prioritizing conservation and management approaches that will benefit future generations. The present research uses a robust model to estimate a severe loss of suitable habitat for *A. penstemonoides* by the year 2050. Additionally, no habitat expansion is expected and the species is unlikely to migrate to track suitable habitat. *Aliciella penstemonoides* may therefore act as a sentinel species for monitoring species threatened by climate change. Management practices may benefit from research targeting seed / pollen dispersal, patch connectivity, and colonization abilities of populations where suitable habitat in 2050 is predicted to overlap with current distributions.
Literature Cited


Peterson, J. S. 1981. Status Report: Gilia penstemonoides. Colorado Natural Heritage Inventory, Colorado Natural Areas Program, Department of Natural Resources, Denver, Colorado, USA.


## Table 2.1: Distribution of *A. penstemonoides* identified in the current research.

<table>
<thead>
<tr>
<th>County</th>
<th>Population</th>
<th>Occurrence</th>
<th>Date Observed</th>
<th>Individual Counts</th>
<th>Management Area / Ownership</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td>1st buttress</td>
<td>6/17/2016</td>
<td>150</td>
<td>GNF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd buttress</td>
<td>6/17/2016</td>
<td>50</td>
<td>GNF</td>
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<tr>
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<td>6/26/2016</td>
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<td>GNF</td>
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<tr>
<td></td>
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<td>fisherman trail</td>
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<td>27</td>
<td>GNF</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Car Killer Crack</td>
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<td>5</td>
<td></td>
<td>GNF</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pioneer Point</td>
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<td>7/9/2016</td>
<td>46</td>
<td></td>
<td>CURE</td>
</tr>
<tr>
<td></td>
<td>East overlook</td>
<td>7/9/2016</td>
<td>12</td>
<td></td>
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</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
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<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Ute</td>
<td>N/A</td>
<td>6/23/2016</td>
<td>240</td>
<td></td>
<td>UTMO</td>
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<td>Road cut</td>
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<td>Climbing site</td>
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<td>Hum_05</td>
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<td>Gateview Trail</td>
<td>6/24/2016</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hinsdale</td>
<td>Fish Canyon</td>
<td>6/24/2016</td>
<td>1</td>
<td></td>
<td>BLM / Private</td>
</tr>
<tr>
<td>Cebolla Creek</td>
<td>Mason Family S.W.A.</td>
<td>6/24/2016</td>
<td>20 (+)</td>
<td>MFSWA / Private</td>
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</tr>
<tr>
<td></td>
<td>Cathedral</td>
<td>6/24/2016</td>
<td>3</td>
<td></td>
<td>BLM / PWA</td>
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<tr>
<td></td>
<td>Spruce Camp Ground</td>
<td>6/25/2016</td>
<td>83</td>
<td></td>
<td>PWA / GNF</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral</td>
<td>Willow Creek</td>
<td>East Willow Cr.</td>
<td>6/8/2016</td>
<td>~100</td>
<td>RGNF</td>
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<td>West Willow Cr.</td>
<td>6/8/2016</td>
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<tr>
<td></td>
<td>Pool Table Road</td>
<td>N/A</td>
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<td>30</td>
<td>RGNF</td>
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<td>Seepage Creek</td>
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<td>7/29/2016</td>
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<td>RGNF</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montrose</td>
<td>Chasm View / Astrolog</td>
<td>7/9/2016</td>
<td>17</td>
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<td>Black Canyon</td>
<td>Dragon Shade Gulley</td>
<td>7/9/2016</td>
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<td></td>
<td>Scenic Cruise</td>
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<td>Checkerboard Route</td>
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<td></td>
<td>BLCA</td>
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<tr>
<td></td>
<td>Atlantis Wall</td>
<td>6/19/2016</td>
<td>25</td>
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<table>
<thead>
<tr>
<th>Site</th>
<th>Rock Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taylor Canyon, Gunnison County</td>
<td>Foliated metagranite</td>
</tr>
<tr>
<td>Black Canyon of the Gunnison, Montrose County</td>
<td>Gneiss</td>
</tr>
<tr>
<td>Big Blue Creek, Gunnison County</td>
<td>Metagranite</td>
</tr>
<tr>
<td>Lake Fork, Gunnison County</td>
<td>Biotite granite</td>
</tr>
<tr>
<td>Cebolla Creek, Hinsdale County</td>
<td>Tuff</td>
</tr>
<tr>
<td>Seepage Creek, Mineral County</td>
<td>Rhyolitic tuff</td>
</tr>
<tr>
<td>Pool Table, Mineral County</td>
<td>Phonolite tuff</td>
</tr>
<tr>
<td>Willow Creek, Mineral County</td>
<td>Andesite</td>
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</table>
Table 2.3: Density estimates (plants per meter$^2$) of *A. penstemonoides*.

<table>
<thead>
<tr>
<th>Population</th>
<th>Estimate</th>
<th>SE</th>
<th>CV</th>
<th>LCL</th>
<th>UCL</th>
<th>DF</th>
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</thead>
<tbody>
<tr>
<td>Black Canyon</td>
<td>0.077</td>
<td>0.06</td>
<td>0.788</td>
<td>0.011</td>
<td>0.514</td>
<td>4.138</td>
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<td>Willow Creek</td>
<td>0.357</td>
<td>0.147</td>
<td>0.412</td>
<td>0.125</td>
<td>1.02</td>
<td>4.54</td>
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<tr>
<td>Taylor Canyon</td>
<td>0.383</td>
<td>0.169</td>
<td>0.443</td>
<td>0.124</td>
<td>1.183</td>
<td>4.462</td>
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<tr>
<td>Pool Table</td>
<td>0.892</td>
<td>0.291</td>
<td>0.327</td>
<td>0.392</td>
<td>2.031</td>
<td>4.914</td>
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<tr>
<td>Lake Fork</td>
<td>2.555</td>
<td>1.301</td>
<td>0.509</td>
<td>0.701</td>
<td>9.307</td>
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</table>

Note: Standard error (SE), coefficient of variation (CV), lower control limit (LCL), upper control limit (UCL), and degrees of freedom (DF).
Table 2.4: Percentage of threats to individual mortality of *A. penstemonoides*.

<table>
<thead>
<tr>
<th>Site</th>
<th>Rock Climbing</th>
<th>Herbivory</th>
<th>Interspecific Competition</th>
<th>Chlorosis</th>
<th>Rock Fall</th>
<th>Dehydration</th>
<th>None</th>
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<td>Willow Creek (n = 59)</td>
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<td>1.69%</td>
<td>0.00%</td>
<td>1.69%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>96.62%</td>
</tr>
<tr>
<td>Pool Table (n = 143)</td>
<td>0.00%</td>
<td>16.08%</td>
<td>0.00%</td>
<td>5.60%</td>
<td>0.00%</td>
<td>1.40%</td>
<td>76.92%</td>
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<td>Black Canyon (n = 57)</td>
<td>10.53%</td>
<td>0.00%</td>
<td>8.77%</td>
<td>0.00%</td>
<td>7.02%</td>
<td>0.00%</td>
<td>73.68%</td>
</tr>
<tr>
<td>Lake Fork (n = 584)</td>
<td>43.15%</td>
<td>0.68%</td>
<td>4.63%</td>
<td>4.63%</td>
<td>0.51%</td>
<td>0.17%</td>
<td>46.23%</td>
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<tr>
<td>Taylor Canyon (n = 72)</td>
<td>56.94%</td>
<td>23.61%</td>
<td>5.56%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>1.39%</td>
<td>12.50%</td>
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Table 2.5: Percent contribution of various environmental predictor variables for predicting potential suitability. See Appendix 2 for definitions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percent contribution</th>
<th>Permutation importance</th>
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<td>Slope</td>
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<td>6.5</td>
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<td>Geology</td>
<td>15.7</td>
<td>4.5</td>
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<td>AHM</td>
<td>15.1</td>
<td>0</td>
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<tr>
<td>Elevation</td>
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<td>61.7</td>
</tr>
<tr>
<td>DD.0</td>
<td>2.7</td>
<td>1.1</td>
</tr>
<tr>
<td>MCMT</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>MAP</td>
<td>1.9</td>
<td>2.7</td>
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<tr>
<td>TRI</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>MSP</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Tave_wt</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Aspect</td>
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<td>0.3</td>
</tr>
<tr>
<td>DD.5</td>
<td>1.4</td>
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<td>Tmax07</td>
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<td>TD</td>
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<td>0.1</td>
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<tr>
<td>PAS</td>
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<td>0.2</td>
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<td>cmiJJA</td>
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</tr>
<tr>
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<td>0.1</td>
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<td>0.1</td>
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<tr>
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Figure Legends

**Figure 2.1:** Locations of *A. penstemonoides* obtained from field research in the summer 2016.

**Figure 2.2:** Percentage of GPS points obtained in the summer of 2016 occurring in ecological system patches according to CNHP 2011.

**Figure 2.3:** Model evaluation statistics averaged from 15 replicates and 25% sample records for testing, (A) Omission vs. predicted area for *A. penstemonoides*, and (B) the area under the ROC curve (AUC).

**Figure 2.4:** Jackknife results of the combined distribution prediction for *A. penstemonoides*. See Appendix 2 for definitions.

**Figure 2.5:** Mean models of predicted habitat suitability for *A. penstemonoides* under (A) 1960-1990 climate, (B) CSIRO_MK3_0 “cool/moist” projected climate for 2050, (C) UKMO_HADCM3 median projected climate for 2050, (D) IPSL_CM4 “warm/dry” projected climate for 2050, and (E) locator map outlining modeled region (red box).

**Figure 2.6:** Results from SDM and projected climate change for the year 2050.
Figure 2.3

A

**Average Omission and Predicted Area for penstemonoides**

Mean area
Mean area +/- one stddev
Mean omission on test data
Mean omission +/- one stddev
Predicted omission

B

**Average Sensitivity vs. 1 - Specificity for penstemonoides**

Mean (AUC = 0.986)
Mean +/- one stddev
Random Prediction
Figure 2.4
CHAPTER 3: SCREENING MICROSATELLITE MARKERS FOR *Aliciella penstemonoides* (POLEMONIACEAE)

Abstract

The Polemoniaceae (Phlox) family has become widely distributed across western North America with a number of endemic taxa becoming restricted to low-competition environments with barren soils. Microsatellite markers developed from the well-studied *Ipomopsis aggregata*, have been shown to have cross-species applicability within the Loeselieae tribe. Genomic DNA extractions of *Aliciella penstemonoides* followed collection of non-destructive genetic samples during the summer of 2016, and were used to test the cross-species applicability of microsatellite markers developed for the distantly related *I. aggregata*. Secondary plant contaminants (i.e., carbohydrates) and the use of primers designed for a distant relative inhibited successful amplification of loci during reactions. However, successful amplification of multiple markers suggests that flanking regions of microsatellites (and traits) within the Polemoniaceae are highly conserved across the Loeselieae tribe.
Introduction

The Loeselieae tribe of the Polemoniaceae family is composed of ca. 100 species from ten genera (Johnson et al. 1996; Porter 1998; Johnson et al. 2008). The genus *Aliciella* is the basal lineage of the Loeselieae tribe and diverged from remaining members near the end of the Paleocene epoch following the Laramide Orogeny and formation of the Rocky Mountains (Foos 1999; Johnson et al. 2008; Porter et al. 2010). The *Giliandra* section of the *Aliciella* subgenera—which contains *A. penstemonoides*—is thought to have diverged from remaining sections by the end of the Oligocene epoch around the time volcanic activity began forming what is now the San Juan Mountains and West Elk Mountains ca. 23 million years ago (mya; Hansen 1965). Near the end of the Tertiary period (ca. five mya), an epeirogenic uplift increased the elevation of the Colorado Plateau and Rocky Mountains by 1,220 – 1,830 meters and allowed the Gunnison River to take its current course where (ca. two mya) it began to rigorously downcut into a Precambrian pluton, known as the Gunnison uplift, at a rate of 30 centimeters per millennium (Hansen 1965; Foos 1999). Habitat for the anticipated center of distribution (i.e., the Black Canyon) therefore appears to have appeared on the landscape relatively late in the evolutionary time line compared to most extant members of the Loeselieae tribe, as does the habitat for the extremely rare species *A. sedifolia* (Brandeg.) J.M. Porter, which is known only to a single alpine scree slope. Compared to other members of the Loeselieae tribe, divergence between extant sister taxa appears to be uncommon during the Pliocene and early Pleistocene era, suggesting that *A. sedifolia* and *A. penstemonoides* are either products of recent speciation, or relict flora that find refuge on the low competition cliff environment (Porter et al. 2010). Microsatellite markers developed for *I. aggregata* must possess highly conserved flanking
regions in order to be effective in the distantly related *A. penstemonoides*. However, trait conservatism within the Polemoniaceae is apparent from the high number of species with a scapiform habit and pinnatifid leaves in a basal rosette—including a fossil record of the extinct *Gilisenium huberi* from the mid-Eocene (Lott et al. 1998).

The extreme environments occupied by members of the *Gilliandra* section of the *Aliciella* genus, impose strong selective forces, which may reduce or eliminate morphological change accompanying speciation and result in a high degree of cryptic species diversity (Porter 1998; Bickford et al. 2007; Trontelj & Fišer 2009). Morphological similarities are apparent throughout all sister species in the *Gilliandra* section, and future studies aimed at a genus level approach to speciation may provide land managers with a better understanding of how similar stress tolerant species will likely respond to future climate scenarios (Bickford et al. 2007; Trontelj & Fišer 2009; Gutiérrez-Tapia & Palma 2016). *A. penstemonoides* appears to be threatened future climate change and is considered moderately vulnerable because of an insular habit and limited seed dispersal ability (Neely et al. 2011). Intraspecific genetic diversity within *A. penstemonoides* may help the species withstand and adapt to novel climate conditions.

Members of the Polemoniaceae (Phlox) family have been used as model species for evolutionary investigations and to advance our understanding of plant speciation, habitat selection, and hybridization (Grant 1959; Wolf et al. 1991; Johnson et al. 2008; Porter et al. 2010). *Aliciella penstemonoides* (M.E. Jones) J.M. Porter (also known as the Black Canyon gilia and *Gilia penstemonoides*) is considered a vulnerable plant (Global Status of G3, Colorado State Status of S3), endemic to cliff habitats of the Upper Gunnison river basin, East-Taylor watershed, Hesadwaters of the Rio Grande, and Uncompahgre River Basin
Due to the obligatory chasmophytic life form, *A. penstemonoides* has been seldomly studied and little is known about species abundance, ecological requirements, or genetic variation (Grey 1982; Porter 1998; Porter & Johnson 2000).

Until recently, cliff systems have been considered ecotones and have not been well studied as independent ecological systems (Larson et al. 2000). A number of endemic Colorado taxa may be described as obligate chasmophytes (e.g., *A. penstemonoides, Argyrochosma fendleri, Draba smithii, D. weberi, Heuchera hallii,* and *Telesonix jamesii*) and grow exclusively in crevices of vertical cliff faces where there is a release from interspecific competition and disturbances such as fire, grazing, land development, off-road vehicle travel, and other threats that commonly place rare Colorado plants at risk (Beatty et al. 2004a; Beatty et al. 2004b; Ladyman 2004; Decker 2006). The vertical habitat and extreme environment create an ecological filter that allows stress-tolerant relict species with poor competitive abilities to persist on cliff ecosystem long after being extirpated in the surrounding landscape (Runemark 1971; Snogerup 1971; Boggess et al. 2017). The severe selective forces imposed by the extreme environmental exposure, in combination with regionally restricted edaphic conditions, result in highly specialized endemic chasmophytic species with narrow distributions and, in theory, a narrower adaptive margin when compared to widespread congener species (Sexton et al. 2009; Bastida et al. 2014).

Endemic obligatory chasmophytes may therefore be occupying the apex of their adaptive margin as selection for phenotypic-environment interactions may outweigh nearly all other selective pressures (Nevo 2012). Genetic marker analyses provide ecologists with the
ability to estimate demographic patterns, and parameters of local adaptation and niche evolution (Selkoe & Toonen 2006).

Microsatellite markers have emerged as a popular method for assessing fine-scale allelic diversity in selectively neutral loci that follow Mendelian inheritance (Selkoe & Toonen 2006). Flanking regions of a microsatellite locus bind to oligonucleotides (primers) that guide amplification during polymerase chain reactions (PCR), and are occasionally known to be highly conserved across taxa (Selkoe & Toonen 2006). The cross-species applicability of primers designed for well-studied species may allow researchers to analyze the genetic diversity of rare relatives, without enduring the economic costs of sequencing and primer development (Squirrell et al. 2003; Selkoe & Toonen 2006).

The current study has two main objectives: 1) screen cross-species applicability of microsatellites developed for *Ipomopsis aggregata* (Pursh) V.E. Grant in *Aliciella penstemonoides* (M.E. Jones) J.M. Porter, and 2) to provide an analysis of presented techniques in hopes of optimizing future genetic extractions and amplifications.

**Methods**

*Collection of genetic material*

During the summer of 2016, I collected 20 non-destructive genetic samples from 14 sub-occurrences of *A. penstemonoides* under appropriate permits from the Black Canyon of the Gunnison National Park (BLCA), Curecanti National Recreation Area (CURE), U.S. Forest Service Rocky Mountain Region (USFS), and U.S. Bureau of Land Management (BLM). I collected individual genetic samples along a vertical gradient by using a rappel rope to access populations of *A. penstemonoides* that occur on vertical rock cliff habitats. Collected
material was no larger than a hole-punch in size and stored in high purity grade silica gel (28-200 mesh; Sigma-Aldrich, St. Louis, MO) until transported to a -80°C freezer at Appalachian State University, Boone, NC, USA.

Genomic DNA extraction and purification

I used Pellet Pestles™ (Fisherbrand™, Hampton, NH, USA) to grind dried and frozen plant samples in a microtube filled with, and ground to the same consistency of, 50-70 mesh sand (Sigma-Aldrich, St. Louis, MO, USA). I extracted genomic DNA from ground tissue samples using PureLink™ Plant Total DNA Purification Kits (Invitrogen, Carlsbad, CA, USA) and DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). A sodium acetate precipitation purified extracted DNA. I measured nucleic acid concentration and contamination using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Microsatellite primer pairs and polymerase chain reactions

Microsatellite primer pairs developed for Ipomopsis aggregata (Pursh) V.E. Grant are shown to have cross-species applicability within the Polemoniaceae family (Wu 2006; Wu et al. 2012). Eleven primer pairs showed successful amplification in A. pinnatifida (Nutt. ex A. Gray) J.M. Porter, a sister species of A. penstemonoides. Integrated DNA technologies (IDT, Coralville, Iowa, USA) synthesized the 11 oligonucleotide pairs developed by Wu (2006) and Wu et al. (2012) with an M13 tag placed on the forward primer. An Eppendorf Mastercycler performed fragment analysis polymerase chain reactions (PCR) under conditions optimized for individual primer pairs. I visually analyzed PCR products by running them in a 1% agarose gel (with 1xTAE buffer and GelRed; Phenix
Research Products, Candler, NC, USA) electrophoresis and imaged using a Gel Doc™ XR+ Gel Documentation System (Bio-Rad, Hercules, CA, USA).

**Results**

A total of 192 individuals from 10 populations underwent genomic DNA extractions and initially yielded low DNA content according to NanoDrop measurements. Imaged extractions in agarose gels showed clear genetic content with occasional residual material in wells and streaking beyond bands. A sodium acetate precipitation significantly increased nucleic concentrations and purity, yet retained poor $A_{260/230}$ ratios regardless of extraction kit.

Five of the primer pairs screened produced successful PCR amplifications with fragments of expected length, while three primer pairs amplified non-specific bands well above 500 base pairs (Table 3.1). Homo- and heterozygosity was observed from imaged gels in primer pair Ipo8, and apparent differences in loci beyond expected fragment base pair lengths (Table 3.1). Optimal thermocycler conditions required protocols that became primer specific, and varying protocols yielded different product patterns under otherwise synonymous reaction conditions (Table 3.2, Appendix 4). Rates of successful amplification from PCR varied both within, and between populations and did present complete data to complete a fragment analysis using fluorophore multiplex dyes.

**Discussion**

I observed morphological variation within and between populations of *A. penstemonoides* and noted a general trend of increasing difference in morphology with increasing distance between populations (Appendix 3). However, isolation may be
occurring in nearby populations due to ecological constraints on patch connectivity and result in neighboring populations taking on drastically different morphological habits. Morphological variation within populations may be a result of high degrees of spatial variability (i.e., surface heterogeneities) and degrees of exposure. Additionally, hybridizations between *A. penstemonoides* and widespread *A. pinnatifida* occur throughout the southern portion of *A. penstemonoides* range. Both *A. penstemonoides* and *A. pinnatifida* produce similar and showy inflorescences that appear to be generally pollinated by flying insects (Grey 1982; Beatty et al. 2004a). *A. penstemonoides* possess small seeds (0.05 mm) that are easily transported by the wind and possess a mucilaginous property when wet (Grey 1982).

The presented research indicates that seven microsatellite primer pairs developed for *I. aggregata* showed successful amplification of target loci when used with species that at the opposite end of the Loeselieae tribe. Irregular success of PCR reactions often occurred within individuals of the same population of *A. penstemonoides*, suggesting that extracted samples may retain carbohydrates or residual phenols that inhibit PCR. Additionally, success rate of PCR appeared to be population dependent and may be explained by poor primer designs for this species. Oligonucleotide Ipo9 was the only primer pair to produce successful amplification of target regions across all reactions within a population. In addition to Ipo9, oligonucleotide pairs Ipo8 and IA370 appear to be the most reliable and successful primers to be used to complete an intraspecific genetic assessment. Oligonucleotide pairs Ipo 186, IA14, IA103, and IA401 require additional work in perfecting thermocycler conditions and further examination of the chemical concentrations of salt in the reactions. Oligonucleotide pairs Ipo109, Ipo180, Ipo190, and
Ipo196 did not produce any successful amplification. All oligonucleotides developed by Wu et al. (2012) produced non-specific bands and yielded different banding patterns depending on thermocycler conditions. Optimal thermocycler conditions for these primers may be further improved, in addition to further examination of MgCl₂ and KCl concentrations.

Microsatellite marker analysis of *A. penstemonoides* may provide researchers with estimates of ecologically important parameters (e.g., relatedness of individuals, local adaption, migratory abilities) that will allow land managers to develop conservation and management practices that align with the species historical adaptive abilities and potential response to future climate change. Different methods of genomic DNA extractions may render cleaner samples that may provide greater success rates of amplification during PCR. Furthermore, continued testing of thermocycler conditions may increase the success rate of amplification in primers producing non-specific bands.
Literature Cited


Peterson, J. S. 1981. Status report: Gilia penstemonoides. Colorado Natural Heritage Inventory, Colorado Natural Areas Program, Department of Natural Resources, Denver, Colorado, USA.


At the rim of the Blue Mesa Dam Overlook, Aliciella penstemonoides retained green basal rosettes throughout the winter. Photograph by Richard Hum in March 2017.
Table 3.1: PCR amplification in individuals of *A. penstemonoides* using microsatellite primer pairs developed for *Ipomopsis aggregata* by Wu (2006) and Wu et al. (2012). Populations listed in descending order of geographic proximity to the theorized central distribution.

<table>
<thead>
<tr>
<th>Population</th>
<th>Ipo8</th>
<th>Ipo9</th>
<th>Ipo109</th>
<th>Ipo180</th>
<th>Ipo186</th>
<th>Ipo190</th>
<th>Ipo196</th>
<th>IA14</th>
<th>IA103</th>
<th>IA370</th>
<th>IA401</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar Point, BLCA</td>
<td>+ / -</td>
<td>+ / -</td>
<td>-</td>
<td>-</td>
<td>+h</td>
<td>-</td>
<td>+h / -</td>
<td>+h / -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pioneer Point, CURE</td>
<td>+ / -</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+h / -</td>
<td>+h / -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine Creek, CURE</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ / -</td>
<td>+h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taylor Canyon</td>
<td>+ / -</td>
<td>-</td>
<td>+h / -</td>
<td>+h / -</td>
<td>+h / -</td>
<td>+h / -</td>
<td>+h / -</td>
<td>+h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring Creek</td>
<td>+ / -</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Big Blue Creek</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+ / -</td>
<td></td>
<td></td>
<td></td>
<td>+ / -</td>
<td>+ / -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Fork</td>
<td>-</td>
<td>+ / -</td>
<td>-</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cebolla Creek</td>
<td>-</td>
<td>+</td>
<td></td>
<td>+h</td>
<td></td>
<td></td>
<td></td>
<td>+ / -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Willow Creek</td>
<td>+ / -</td>
<td>+</td>
<td>+</td>
<td>+ / -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ / -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool Table</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td>+ / -</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Note: + = successful amplification in all tested individuals; +h = amplification of high molecular weight product; + / - successful amplification in a portion of tested individuals; - = no amplification.
Table 3.2: Thermocycler protocols resulting in optimal amplification of microsatellite primer pairs in *A. penstemonoides* during PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Thermocycler protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipo8</td>
<td>WU2006td3</td>
</tr>
<tr>
<td>Ipo9</td>
<td>WU2006td60</td>
</tr>
<tr>
<td>Ipo109</td>
<td>WU2006td3</td>
</tr>
<tr>
<td>Ipo186</td>
<td>WU2006td3</td>
</tr>
<tr>
<td>IA14</td>
<td>Wu2012-103</td>
</tr>
<tr>
<td>IA103</td>
<td>Wu2012-103</td>
</tr>
<tr>
<td>IA370</td>
<td>Wu2012td</td>
</tr>
<tr>
<td>IA401</td>
<td>Wu2012-103</td>
</tr>
</tbody>
</table>
Appendix 1: Beanplots for (A) hourly temperature and (B) light intensity recordings from Hobo Pendant Temperature Light Data Loggers (Onset Computer Corporation, Bourne, MA) recorded from July 4 – 28, 2016. Dotted black bar indicated overall mean while black bar on beans indicate sample mean. Maximum temperature recordings are likely inflated from maximum greenhouse effects.
Appendix 2: Bioclimatic environmental predictor variables used in the MaxEnt SDM.

**Monthly variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmin</td>
<td>minimum temperature for January (°C)</td>
</tr>
<tr>
<td>Tmax07</td>
<td>maximum temperature for July (°C)</td>
</tr>
<tr>
<td>PPT</td>
<td>total precipitation for a given month (mm)</td>
</tr>
</tbody>
</table>

**Bioclimatic variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT</td>
<td>mean annual temperature (°C)</td>
</tr>
<tr>
<td>MCMT</td>
<td>mean temperature of the coldest month (°C)</td>
</tr>
<tr>
<td>MWMT</td>
<td>mean temperature of the warmest month (°C)</td>
</tr>
<tr>
<td>TD</td>
<td>Difference between MCMT and MWMT, as a measure of continentality (°C)</td>
</tr>
<tr>
<td>MAP</td>
<td>mean annual precipitation (mm)</td>
</tr>
<tr>
<td>MSP</td>
<td>mean summer (May to Sep) precipitation (mm)</td>
</tr>
<tr>
<td>AHM</td>
<td>annual heat moisture index, calculated as (MAT+10)/(MAP/1000)</td>
</tr>
<tr>
<td>SHM</td>
<td>summer heat moisture index, calculated as MWMT/(MSP/1000)</td>
</tr>
<tr>
<td>DD.0</td>
<td>degree-days below 0°C (chilling degree days)</td>
</tr>
<tr>
<td>DD.5</td>
<td>degree-days above 5°C (growing degree days)</td>
</tr>
<tr>
<td>NFFD</td>
<td>the number of frost-free days</td>
</tr>
<tr>
<td>bFFP</td>
<td>the julian date on which the frost-free period begins</td>
</tr>
<tr>
<td>eFFP</td>
<td>the julian date on which the frost-free period ends</td>
</tr>
<tr>
<td>PAS</td>
<td>precipitation as snow (mm)</td>
</tr>
<tr>
<td>EMT</td>
<td>extreme minimum temperature over 30 years</td>
</tr>
<tr>
<td>Eref</td>
<td>Hargreave's reference evaporation</td>
</tr>
<tr>
<td>CMD</td>
<td>Hargreave's climatic moisture index</td>
</tr>
<tr>
<td>CMI</td>
<td>Hogg's climate moisture index</td>
</tr>
<tr>
<td>cmiJJA</td>
<td>Hogg's summer (Jun to Aug) climate moisture index</td>
</tr>
<tr>
<td>Tave_wt</td>
<td>winter (Dec to Feb) mean temperature (°C)</td>
</tr>
<tr>
<td>Tave_sm</td>
<td>summer (Jun to Aug) mean temperature (°C)</td>
</tr>
<tr>
<td>PPT_wt</td>
<td>winter (Dec to Feb) precipitation (mm)</td>
</tr>
<tr>
<td>PPT_sm</td>
<td>summer (Jun to Aug) precipitation (mm)</td>
</tr>
</tbody>
</table>
Appendix 3: Morphological variations of *A. penstemonoides* across its range. Colored points on map correspond to colored point in bottom right of image. Photographs by Richie Hum.
## Appendix 4: Conditions for Eppendorf Mastercycler.

<table>
<thead>
<tr>
<th>Thermocycler Header</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Wu2006td3</td>
<td>96°</td>
</tr>
<tr>
<td></td>
<td>2:00</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>***decline 0.5° per cycle</td>
</tr>
<tr>
<td>Wu2006td60</td>
<td>96°</td>
</tr>
<tr>
<td></td>
<td>2:00</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>***decline 0.5° per cycle</td>
</tr>
<tr>
<td>Wu2012-103</td>
<td>94°</td>
</tr>
<tr>
<td></td>
<td>4:00</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>***decline 1° per cycle</td>
</tr>
<tr>
<td>Wu2012td</td>
<td>94°</td>
</tr>
<tr>
<td></td>
<td>4:00</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>***decline 1° per cycle</td>
</tr>
</tbody>
</table>
Vita

Richard Auston Hum was born in Vail and raised in Breckenridge, Colorado, where he developed an inherent love for outdoors from hiking, skiing, and camping with his family. He graduated from Fort Lewis College in Durango, CO, in 2014 after focusing on Colorado Native plant identification. In the fall of 2015, he joined the Cliff Ecology Lab at Appalachian State University where he began integrating his rock climbing experience with academic studies to earn his Master of Science degree, which was awarded in August 2017.

The author finds easy access to a cluster of *Aliciella penstemonoides* below an overlook in the Black Canyon of the Gunnison National Park during the summer of 2016. Photograph by Kate Lis.