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CONSERVATION AND MANAGEMENT OF THE HIGH ELEVATION SUBSPECIES OF THE GUNNISON'S PRAIRIE DOG, *CYNOMYS GUNNISONI GUNNISONI*

by

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Conservation and Management of the High Elevation Subspecies of the Gunnison's Prairie Dog, *Cynomys gunnisoni gunnisoni*

Thesis directed by Dr. Andrew P. Martin

ABSTRACT

Species of conservation concern often exist in fragmented habitats and experience low population numbers. Habitat fragmentation can separate groups of individuals into small demographically independent populations, which are susceptible to evolutionary processes like genetic drift, which can erode genetic diversity and reduce the adaptive potential of a population. Maintaining large population numbers, conserving genetic variation, and preserving population connectivity is important for the long-term persistence of populations and for the exchange of individuals and genetic diversity between populations. The focus of my research is on *Cynomys gunnisoni gunnisoni,* a subspecies of Gunnison's prairie dog that inhabits the montane regions of south-central Colorado and north-central New Mexico. Today, *C.g. gunnisoni* exists in a patchwork of colonies among large mountain valleys and basins separated by impassable mountain ranges. In my dissertation research, I evaluated how populations of *C.g. gunnisoni* are connected across the landscape, used genetic information to evaluate population structure, and characterized genetic diversity among colonies and populations. In Chapter 1 I address how conservation actions are needed to stem the loss of biodiversity. Some species, such as prairie dogs in the genus *Cynomys*, are keystone species and ecological engineers whose presence and actions have disproportionately large impacts on ecosystems. The large reduction in prairie dog population numbers has negatively impacted species and ecosystems in the Great Plains and Rocky

Mountains. Conservation actions that benefit prairie dogs could have cascading effects that benefit habitats and the species that interact with prairie dogs such as the burrowing owl, mountain plover, and black-footed ferrets. *C.g. gunnisoni* is of conservation concern due to low population sizes and because it inhabits a fragmented landscape of the southern Rocky Mountains. Better understanding the population dynamics and conservation concerns of *C.g. gunnisoni* can inform on conservation management actions for the subspecies. In Chapter 2, I use landscape genetics methods with a circuit theory modeling approach to investigate how the landscape influences dispersal patterns and population distributions of the subspecies. I found that distance, extreme elevations, sloping terrain, and forest, aquatic, and urban habitats are expected to resist population connectivity. The conversion of habitat for agriculture and food production may be important in altering dispersal paths away from areas which would otherwise promote prairie dog dispersal. Large mountain ranges act to separate the subspecies' range into multiple population areas while drainage systems may provide habitat corridors that allow prairie dogs to colonize marginal habitat space in mountainous terrain. In Chapter 3, I used multiple methods of genetic clustering and phylogenetic networks to investigate the genetic structure of *C.g. gunnisoni* and inform on the establishment of management units for the subspecies. I identified at least three genetic groups within the subspecies. Colonies within major watersheds generally cluster together more than do colonies from different watersheds. I observe there is high genetic differentiation between most colonies that are not geographically near (within a few kilometers of each other) and little gene flow occurs across large distances. I also found that genetic diversity is overall low within colonies, though unique allelic variation may be harbored in colonies that are isolated or at the edges of occupied habitats. The conservation of *C.g. gunnisoni* may benefit from management actions that focus on elevating the genetic connectivity of prairie dog populations to prohibit reduction of population

fitness by genetic deterioration and other processes, perhaps using assisted migration strategies. In my last Chapter, I presented a remote learning lesson in population and conservation biology with the Northern Spotted Owl. In this lesson I present a lab that utilizes Google Earth Web to create a virtual simulation where students can perform a “mark-recapture’ exercise and collect data to conduct a demographic analysis of how a population of Northern Spotted Owls is doing. This lesson introduces students to population biology, conservation management, the use of demographic data and only requires internet access to be conducted remotely or independently.

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CHAPTER 1

INTRODUCTION

Loss of biodiversity is occurring at levels similar to mass extinctions observed in the fossil record (Jablonski 1994; Peters and Foote 2002); indeed, we are in the midst of Earth's sixth great mass extinction event (Barnosky et al. 2011). Anthropogenic activities that impact the Earth's biomes are largely to blame (Leakey and Lewin 1996). Hunting, eradication, pollution, habitat degradation, and the loss of demographic connectivity across species' ranges have contributed to the decline of biodiversity and extinction of species (Haddad et al. 2015; Newbold et al. 2015). The increasing rate of biodiversity loss due to human activities and human mediated climate change has been growing in appreciation and it is apparent that dedicated efforts must be made to reduce the extinction of species and loss of ecosystems around the world (Fahrig 2003; Bellard et al. 2012; IPBES 2019).

Conservation biology is a "mission-oriented crisis discipline" (Soule 1985) developed in response to the loss of biodiversity (Bellard et al. 2012; IPBES 2019). As human populations grow, more natural habitat is being lost or converted for other purposes (Hoekstra et al. 2005). Natural lands are being urbanized, utilized for agricultural purposes, or degraded for natural resource attainment and has led to the loss, degradation, and fragmentation of habitats and ecosystems (Fahrig 2003). Increasingly, human activities are affecting species and ecosystems around the world, creating conservation needs for a growing number of species and habitats (Hanski 2011). For example, in the late 1900's, many species of raptors were declining due to habitat destruction and degradation, illegal shooting, and a pesticide (DDT) that contaminated their food and reduced reproductive success (Grier 1982). Government protections, recovery programs, and a ban on DDT

resulted in the recovery of many species of raptors, including the bald eagle, which was removed from the Endangered Species List in 1995 (USFWS 2007). Another conservation program focused on the black-footed ferret. At one point thought extinct, the species has increased in population numbers through a captive breeding program and reintroduced individuals back into the wild (Garelle et al. 2012). Due to extremely low numbers of surviving individuals, inbreeding, and low genetic diversity still afflict the species, but recently researchers have experimented with increasing genetic diversity by cloning ferrets using genomes collected from specimens that had been frozen and preserved in biological archives (Santymire et al. 2014; Sandler et al. 2021). Breeding cloned ferrets with captively bred individuals will introduce genotypes that were once lost from the species. Each conservation issue requires its own evaluation and development of management actions. It is thus important to develop effective strategies to research, evaluate, and monitor species that are in conservation need, and then develop effective management strategies that will provide short- and long-term conservation results.

All species have their niche in the ecosystem, but the activities of some species have disproportionately large effects on the ecosystem and the species in it (Delibes-Mateos et al. 2011; Hale and Koprowski 2018). These species are known as “keystone” species: those that maintain the organization and function in their communities by directly or indirectly influencing other species or the ecosystem (Paine 1969; 1995; Kotliar 2000). In western North America, wolves are an example of a keystone species; maintaining viable populations of wolves on the landscape has had direct effects on how prey species behave, and thus indirectly affecting the ecological community within the environment (Ripple and Larsen 2000; Fortin et al. 2005). Keystone species like wolves facilitate increased community level diversity and enhance ecosystem services and productivity in regions where they have been reestablished (Ripple and Beschta 2012).

In some cases, the activities of species modify the landscape, which can affect the survival of other species (Mills et al. 1993). The actions of these "ecological engineers" change biotic or abiotic factors on the landscape, altering habitats that can be used by the ecological engineer or other species (Jones et al. 1994). Many rodents have been identified as keystone species and of these, a few are also considered ecological engineers (Reichman & Seabloom 2002; Zhang et al. 2003). Beavers are well known modifiers of the landscape. Beavers change the ecology and hydrology of stream ecosystems as their activities lead to the formation of ponds and slow-moving drainage systems that benefit willow communities and increase species richness on the landscape (Naiman et al. 1986; Wright et al. 2002; Rossell et al. 2005). As beavers engineer riparian regions in western North America, prairie dogs engineer grasslands and shrublands in the plains and mountain ecosystems of western North America (Jones et al. 1994; Bangert and Slobodchikoff 2000). Each prairie dog creates and maintains burrows, which contributes to the turnover of soil. These burrowing activities cycle nutrients above and below ground, store carbon in the soil, decrease the rate of erosion, and contribute to better drainage of grassland soils (Kotliar et al. 1999; Bangert and Slobodchikoff 2000; Martínez-Estévez et al. 2013; Brazier et al. 2021). Populations of prairie dogs lead to more fertile soils, contributing to healthier vegetation, and an increase of nutritional plant biomass and primary and secondary activity (Martínez-Estévez et al. 2013).

The prairie dog:

Prairie dogs and the habitat they create provide direct and indirect benefits to many other species. The endangered mountain plover (*Charadrius montanus*) nests in grasslands with short and sparse vegetation. Prairie dogs forage and clip grasses and forbs, resulting in good habitat for nesting mountain plover compared to grasslands off prairie dog colonies. (Dinsmore et al. 2005; Duchardt et al. 2020). Burrowing Owls (*Athene cunicularia*) use ready-made burrows, often

created by prairie dogs, and generally nest in occupied colonies (Duchardt et al. 2020). Prairie dogs are also a food source for numerous predators including species of raptors and foxes (*Vulpes* sp.), coyotes (*Canis latrans*), bobcat (*Lynx rufus*), American badgers (*Taxidea taxus*), and the endangered black-footed ferret (*Mustela nigripes*) (Davidson et al. 1999; Lomolino & Smith, 2004; Hoogland 2013). Importantly, prairie dogs contribute to landscape heterogeneity, maintaining patches of varying habitat that benefits certain species (Duchardt et al. 2022). Historically, prairie dogs were abundant, and colonies could contain millions of individuals; thus, resulting in large effects of prairie dogs as a keystone species and ecosystem engineer. Today, these effects are limited by low population numbers and reduced amount of occupied habitat.

Prairie dog colonies consist of numerous family groups, called clans, each of which creates and maintains multiple burrows and burrow systems (Fitzgerald and Lechleitner 1974; Halpin 1987; Hoogland 1995; Hoogland 1999). A colony is made up of many prairie dog clans that occupy discrete areas of the habitat (Kotliar et al. 2006). Colony growth can occur through reproduction, though prairie dogs are considered to have low population growth (relative to many other small mammals). Female prairie dogs reproduce only once each year, weaning just 3-5 pups per litter, and 50% to 70% of those pups do not make it past their first year (Hoogland 2001; Farid 2019; Minnig and Hoogland 2020). Dispersal from other colonies can lead to immigration supplementing colony numbers or the recolonization of unoccupied habitat (Haplin 1987; Roach et al. 2001).

Prairie dogs were once abundant among the Great Plains and Rocky Mountain regions of North America, but populations have seen a dramatic decline in the last few centuries (Miller & Ceballos 1994). Prairie dog habitats have been converted for the purposes of agriculture, ranching, urbanization, and resource extraction (Seglund and Schnurr 2010). Often considered a pest to farmers and ranchers that settled the Great Plains and Rocky Mountain regions, mass eradication

campaigns were implemented to remove large populations of prairie dogs from areas valued for farming and ranching (Miller et al. 1990). In addition to these factors, the plague, a bacterial pathogen, is considered the primary threat to existing prairie dog populations (Cully et al. 1997). *Yersinia pestis,* which is the causal agent of the bubonic and sylvatic plague, is endemic to central Asia and was introduced to North America around 1900 and has since invaded regions occupied by prairie dogs (Wherry 1908; Barnes 1993). Habitat loss, extermination, plague, and other factors have led to a 99% reduction in prairie dog habitat occupancy across their historical range (Miller & Ceballos 1994; Kotliar et al. 2006).

Prairie dogs are at risk from a multitude of different factors. While predation, extreme climate events, and shooting can all negatively affect population numbers these rarely result in the loss of all individuals from large colonies. In contrast, habitat conversion can lead to a permanent loss of habitat space, and plague can cause catastrophic loss of the animals. Habitat loss and plague can lead to total colony extirpation (Cully and Williams 2001; Roach et al. 2001). When prairie dog colonies are lost, vegetation will begin to revert to that which resembles the surrounding environment, vegetation will grow higher, burrows will gradually fill in, and species like black-footed ferret, badgers, burrowing owls, and mountain plover will decrease in abundance (Duchardt et al. 2020). Since many species benefit from the conservation of prairie dog populations some conservation biologists have worked to reestablish prairie dog colonies. The reestablishment of prairie dogs onto extirpated colonies has seen a return of the species that have been known to associate with prairie dog colonies (Davidson et al. 2018). Thus, prairie dog conservation is vital for conservation of other species.

Currently, sylvatic plague is the most concerning threat to population persistence (Wagner et al. 2006). Plague can spread through direct contact of infected mammals or transmitted by

infected fleas (Barnes 1982, 1993; Biggins and Kosoy 2001). When plague is introduced into a colony, it can spread rapidly among individuals and cause mortality rates of 90%-100% (Lechleitner et al. 1962, 1968; Rayor 1985; Cully 1989; Cully and Williams 2001; Colman et al. 2021). Ecke and Johnson (1952) documented a population of Gunnison's prairie dogs in South Park, CO that occupied 914,00 acres in 1941. Sylvatic plague entered the region in 1947 and within two years, over 95% of the prairie dogs were extirpated from South Park. Another consequence of sylvatic plague is that large well connected colony complexes are reduced to multiple small and fragmented colonies. Keuler et al. 2020 observed that after plague entered a large colony complex of black-tailed prairie dogs in South Dakota, large colonies were decimated, and prairie dogs were removed from most of the occupied habitat. The number of colonies increased but were much smaller and fragmented and had greater inter-colony distances. Across prairie dog systems, plague and other factors have resulted in the reduction of occupied area, numbers, and connectivity of prairie dog populations.

Prairie dog populations function as a metapopulation, consisting of occupied and unoccupied patches of habitat distributed across a habitat matrix (Levins 1969; Harrison and Taylor 1997; Hanski 1999; Antolin et al. 2002, Stapp et al. 2004, Salkeld et al. 2006, Snäll et al. 2008). Dispersal connects habitat patches and patch occupancy depends on the dynamic balance between colony extinction and colonization (McCullough 1996; Lidicker and Koenig 1996; Hanski 1999; Antolin et al. 2006). Metapopulation dynamics are dependent on the dispersal of individuals between suitable patches across a conductive habitat space (Lidicker and Koenig 1996; Hanski 1999; Roach et al. 2001). Metapopulation persistence is dependent on rates of colonization equaling or exceeding rates of local extinction (Levins 1969; McCullough 1996; Hanski 1999). Individual dispersal and a conductive habitat matrix are essential for maintaining prairie dog

metapopulation structure (Fahrig and Merriam 1994; Hanski 1999; Hanski and Simberloff 1997; Harrison and Taylor 1997). The effects of sylvatic plague have led to prairie dog populations that now more often occur in small colonies separated by uninhabited landscape but can be connected through dispersal (Roach et al. 2001).

The Gunnison's prairie dog:

The Gunnison's prairie dog (*Cynomys gunnisoni*) is one of five extant species in the genus *Cynomys* and is the only one with recognized subspecies, *C.g. gunnisoni* and *C.g. zuniensis* (Figure 1.1). In 2008, the USFW reviewed the Gunnison's prairie dog for listing under the ESA and found that the populations in the montane portion of the Gunnison's prairie dog range were in decline and determined the montane portion of the Gunnison's prairie dog range warranted listing under the Endangered Species Act (ESA), but protections were precluded due to other species being of higher conservation priority (USFWS 2008; 2013). This designation prompted additional research and conservation actions from agencies who managed species and lands that were included in *C.g. gunnisoni's* range. Plague management programs were implemented to protect targeted colonies (Seglund and Schnurr 2010). Burrow dusting -a term that describes the use of insecticides that are sprayed into prairie dog burrows in order to kill fleas- was used as an initial conservation strategy to proactively reduce the risk of colony loss from sylvatic plague (Seery et al. 2003; Tripp et al. 2016). In more recent years, a sylvatic plague vaccine (SPV) delivered to prairie dogs in the form of baits, has been shown to reduce the risk of colony extirpation and is currently the primary conservation method, though burrow dusting is still used (Rocke et al. 2008; 2017, Tripp et al. 2017, Seglund et al. 2022).

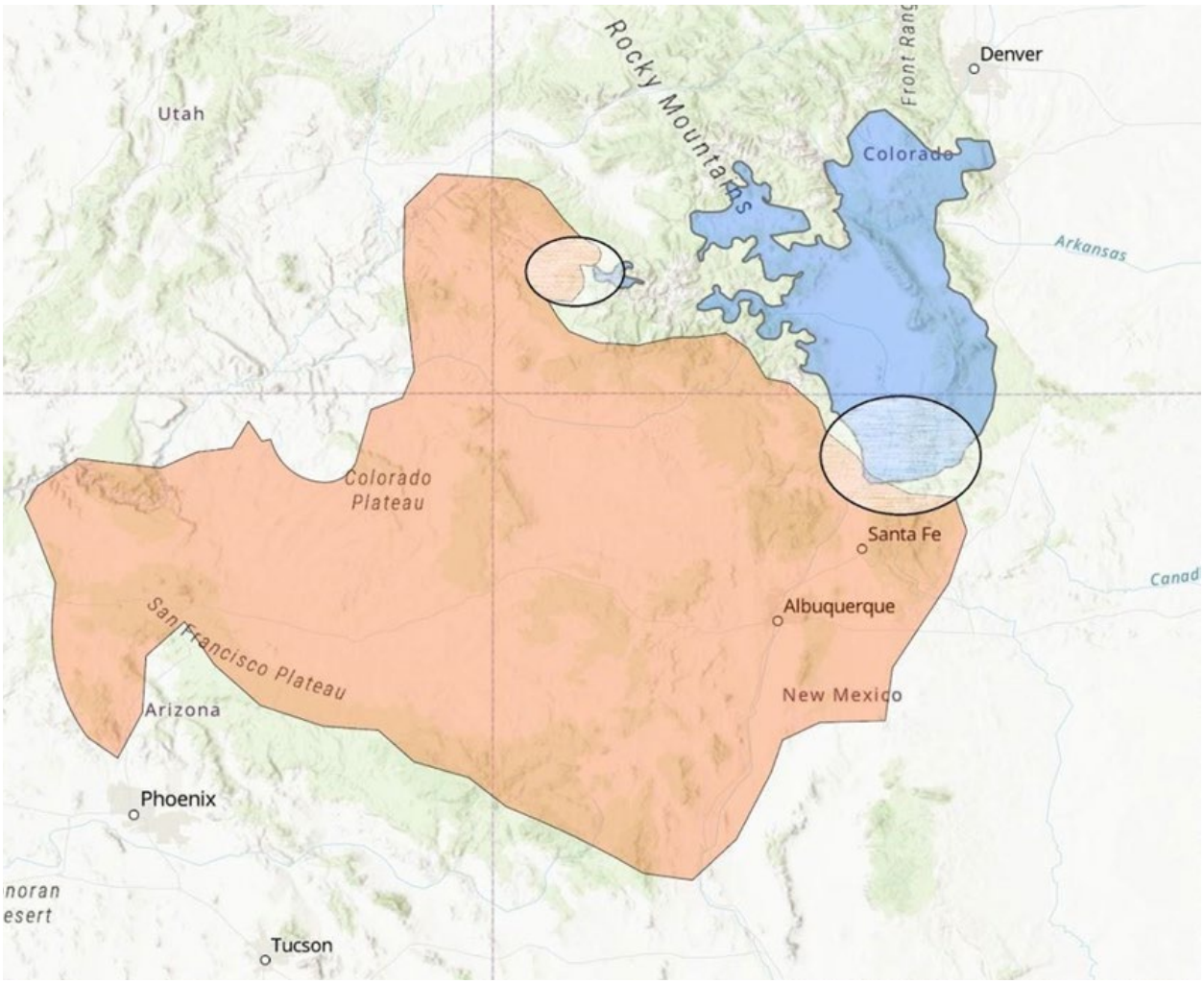


Figure 1.1: The approximate range of *Cynomys gunnisoni*. *C.g. zuniensis* subspecies range is in orange and *C.g. gunnisoni* is in blue. Two identified contact zones exist with evidence of hybridization between the subspecies. These two areas are circled and represent uncertainty in delineating subspecies boundaries.

After the USFWS listing decision in 2008, research was conducted to investigate the existence and distribution of the two proposed subspecies. This research used genetic, physiological, and environmental data to argue for the designation of two subspecies of Gunnison's prairie dogs and refined the expected range boundaries of each (Sackett et al. 2014). This information resulted in the range of *C.g. gunnisoni*, already much smaller than that of *C.g. zuniensis,* being reduced further as much of the populations in New Mexico that were thought to be *C.g. gunnisoni* were in fact genetically more similar to *C.g. zuniensis*.

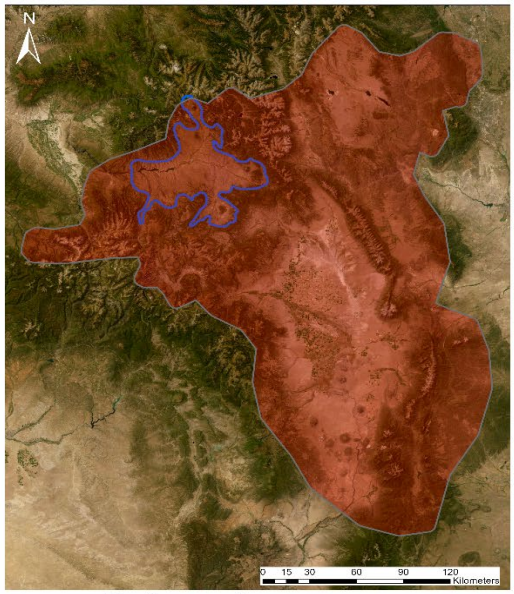
Another review was conducted in 2013 to reevaluate Gunnison's prairie dog as a candidate species under the ESA. The genetics research and the conservation work that occurred since the 2008 decision was considered in this review, which officially recognized the establishment of two subspecies of Gunnison's prairie dogs for the first time and deemed that neither *C.g. zuniensis* or *C.g. gunnisoni* was warranted protection under the ESA. The 2013 listing decision cited the ongoing conservation work, primarily the annual plague management activities, as an important reason for not listing the species at the time (USFWS 2013). While ESA protections were not warranted, the Gunnison's prairie dog remains a species of conservation concern (USFWS, 2008; *WildEarth Guardians v. Salazar,* 2009; USFWS, 2013; *WildEarth Guardians v. Jewel*, 2015) and *C.g. gunnisoni* is of particular concern as it has a smaller range, lower population numbers, and inhabits a more fragmented habitat compared to *C.g. zuniensis* (USFW 2013).

Populations of *C.g. gunnisoni* are likely to occur in a hierarchical structure. Herein, I will use terms to describe different levels of populations (Table 2.2). Within *C.g. gunnisoni* are two subspecies, *C.g. zuniensis* and *C.g. gunnisoni*. *C.g. gunnisoni* occupies many large mountain valleys, such as the San Luis Valley, Gunnison Bains, and South Park (Streich 2018). These large habitable areas and the marginal habitat surrounding them are considered part of a "population

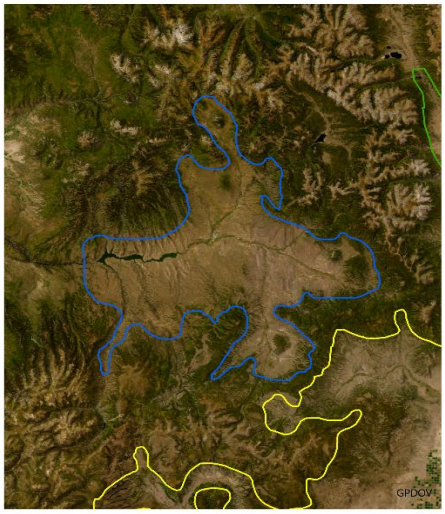
area". This is a loosely defined term to designate an area that, prior to population losses, would likely have consisted of many colonies that were connected to each other through dispersal and separated from each other by landscape barriers such as mountain ranges. With each population area, there are clusters of colonies that are in close geographic proximity, but separated from other clusters by uninhabited habitat space that spans distances of more than 5 or 10 km. These colony clusters would be expected to have dispersal occurring among colonies within a cluster than between clusters. A colony is a discrete patch of habitat occupied by prairie dogs.

Table 1.2: Overview of population hierarchy with terms used in this paper.

Unit	Definition	Meaning
Species	*C. gunnisoni*	The species
Subspecies	*C.g. gunnisoni* *C.g. zuniensis*	Large evolutionary divergence between groups of individuals
Population area	Large area that is mostly inhabited by prairie dogs and geographically separated from other areas.	Areas such as South Park, Gunnison Basin, San Luis Valley and the surrounding areas within each watershed
Colony Clusters	A group of colonies that are geographically proximate and experience high dispersal and gene flow.	Colonies located in a network and dispersal occurs regularly between colonies.
Colony	A discrete occupied area on the landscape containing prairie dogs.	The smallest population unit.

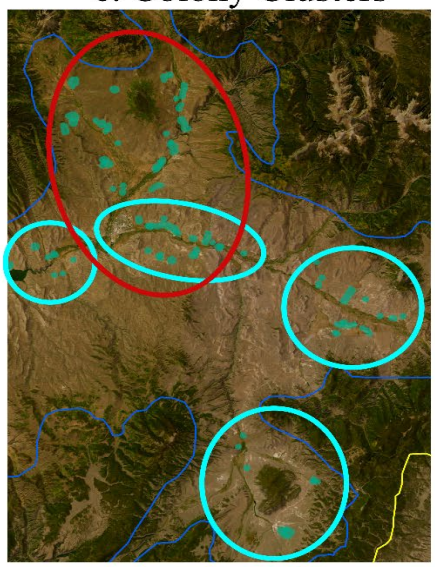


a. Subspecies



b. Gunnison Basin: A popualtion area within a watershed

c. Colony Clusters



d. Colonies

Figure 2.2: Examples of population hierarchy for Gunnison's prairie dogs: a) An outline of the approximate range of *C.g. gunnisoni.*_b) One of the large population areas within the subspecies range. A population area will be simplified to the areas inhabited by C.g. gunnisoni in the major watersheds of the Arkansas, South Platte, Rio Grande, Gunnison, and San Miguel River systems. c) Colony clusters are colonies that are close in geographic proximity and are expected to experience gene flow. Cyan circles represent clusters if dispersal is limited to small distances, while the red circle shows a potential cluster if dispersal is high across larger distances. d) The smallest population unit is the colony, a discreet area on the landscape that is continuously inhabited by prairie dogs.

In this dissertation, I characterized population structure, connectivity, and genetic variation of *C.g. gunnisoni.* I hypothesized that mountain ranges act as barriers to gene flow and that genetic differentiation would be lower among colonies located within mountain basins -characterized by watershed drainages- than among colonies located in different watersheds. Colonies that exist in the peripheral areas with low connectivity showed elevated levels of genetic differentiation and collectively harbored more of the unique genetic diversity found in this subspecies than colonies in more central locations that were relatively well connected by dispersal. Low gene flow between colonies may have allowed for the accumulation of unique alleles, such that weakly connected portions of a metapopulation act like reservoirs retaining unique genetic diversity.

In *C.g. gunnisoni*, there was a high degree of genetic structure with genetic groups mostly occurring in separate watersheds. There was evidence of population substructure, particularly within the Rio Grande watershed and to a lesser extent in the Gunnison Basin. I also found that large mountain ranges inhibited gene flow, but some low elevation areas along mountain ranges could be likely corridors for interpopulation gene flow. Colonies that were expected to be isolated showed high levels of genetic differentiation and in most cases also had high levels of unique genetic diversity. To maximize the preservation of the most genetic variation across the subspecies range, isolated populations and colonies could be considered for increased conservation attention. Management actions that work to maintain colony occupancy across the subspecies range, promote colony connectivity, and increase gene flow between colonies should be considered. Increasing connectivity and gene flow can allow for adaptive allelic variation that resides in unconnected population to be distributed across more colonies.

The conservation of the Gunnison's prairie dog is an example of one of many species that have experienced large losses in population numbers or available habitat. Biologists and managers are working to conserve many species and ecosystems around the world. An important aspect of conservation is the education of the public and students whose decisions will impact the future of global biodiversity. In addition to the described dissertation, I developed a virtual learning lesson focused on population biology and the conservation of the Northern Spotted Owl. This course module was designed to provide an introduction for undergraduate students in population ecology using the Google Earth Web platform. This lesson is designed to adapt an already existing lab to a virtual environment to facilitate student learning away from the classroom, as was the case in the recent COVID-19 pandemic.

CHAPTER 2

POPULATION CONNECTIVITY OF GUNNISON'S PRAIRIE DOGS IN A HETEROGENEOUS LANDSCAPE

2.1 ABSTRACT

The movement of individuals throughout a species' range maintains demographic cohesion, limits localized inbreeding, and facilitates colonization and establishment in new or extirpated locations. Understanding the functional connectivity of populations is key to their management and conservation; however, it is sometimes difficult to record the movement of individuals, particularly those that are small and cryptic. Indirect methods (e.g., genomic and spatial information) can be used for inferring how gene flow connects populations. In this study, I obtained genetic and landscape information to discover how the complex landscape of the southern Rocky Mountains influences dispersal patterns of a social rodent, the Gunnison's prairie dog (*Cynomys gunnisoni gunnisoni*). Using an isolation-by-resistance modeling framework, certain habitat types, slope, elevation, and geographic distance show evidence of reducing gene flow. The resulting resistance model characterized connectivity across the subspecies range and predicted paths of intra and inter population connectivity. Mountain valleys provide large expanses of connected habitat, while mountain ranges separate populations. Wide mountain valleys and basins provide large regions of well-connected habitat for *C.g. gunnisoni*. Isolation of some colonies can occur when colonies exist near the edges of habitable areas or along narrow and fragmented habitat corridors.

2.2 INTRODUCTION:

Ensuring population connectivity is increasingly recognized as essential to the conservation of species (IUCN 2017; Crooks and Sanjayan 2006). Dispersal facilitates species distribution, the movement of individuals from large to small populations, and the exchange of genetic variation between populations (Dickson et al. 2019). Without dispersal, small populations are vulnerable to stochastic processes that can lead to the erosion of genetic diversity, a reduction of population fitness, and extirpations (Frankham 1996). However, it remains difficult to understand and measure the degree in which populations are connected and how landscape patterns affect gene flow (Koenig et al. 1996; Lowe et al. 2010).

Habitat quality and landscape features influence how individuals are distributed across a species' range (Manel et al. 2003; Storfer et al. 2007). Often, the central portion of a population contains high quality habitat and as the distance increases from the population center, the quality of that habitat will decrease, becoming increasingly marginal and will support fewer individuals (Eckert et al. 2008; Micheletti and Storfer 2015; Trumbo et al. 2016). Edge populations will also typically experience higher levels of genetic differentiation compared to core communities. This is because higher gene flow will act to homogenize the gene pool in central populations and overcome the effects of genetic drift, which acts more strongly on edge populations with lower population connectivity (Eckert et al. 2008; Trumbo et al. 2016).

In the Rocky Mountains western North America, the montane subspecies of Gunnison's prairie dog (*Cynomys gunnisoni gunnisoni*) occupies high quality habitat areas in mountain valleys as well as in peripheral habitat patches. Colonies are distributed across population areas, with most occurring in high quality grassland and shrubland habitat in large open basins and valleys, but

many also occur in montane meadows along river corridors and in montane meadows found in forest ecosystems (Seglund et al. 2005; Seglund and Schnurr 2010). Multiple population areas contain a large central habitat core, usually a large mountain valley or basin that supports many prairie dog colonies. South Park, the Gunnison Basin, and the San Luis Valley are three such areas that provide an abundance of good quality prairie dog habitat. Each of these mountain valleys is surrounded by hills and mountains, where prairie dog habitat becomes increasingly marginal, but prairie dogs often occur in lower densities.

Gunnison's prairie dogs exist in a hierarchical population structure. Individual prairie dogs live in family groups, each referred to as a clan (Fitzgerald and Lechleitner 1974; Halpin 1987; Hoogland 1995; Hoogland 1999) that consists of a small set of related individuals that defend a territory consisting of a subset of burrows on a colony (Martínez-Estévez et al. 2013). A colony consists of multiple clans that occupy a discrete habitat space (Johnson and Collinge 2004). A metapopulation consists of habitat patches that are connected across a discontinuous habitat space by dispersal (Levins 1969; Hanski 1999; Roach et al. 2001). Metapopulation structure stabilizes a reginal persistence as dispersal can allow for the colonization of unoccupied habitat patches or the recolonization of unoccupied habitat patches (Hanski 1999; Levins 1969; Johnson and Collinge 2004). Here, the term -colony- is used to represent a discrete local population of prairie dogs that occupy a habitat patch in a nearly continuous manner. The term -colony cluster- will represent a number of colonies that are in close geographic distance to each other and are expected to be connected through moderate or high levels of dispersal. The term -population area- is used to indicate a network of habitat patches that show evidence of being connected through potential dispersal paths in recent history, or what may have been considered a large metapopulation prior to population decline in the 1900's (Fitzgerald and Lechleitner 1974; Rayor 1985 & 1988).

The conversion of natural habitat to urban development and agriculture has occurred throughout the Gunnison's prairie dog range (Knowles 2002; Seglund et al. 2005). However, the percent of lands that have been converted from natural habitat within the subspecies range is low, around 2% for urbanization and 3% for agriculture (Seglund et al. 2005), but much of this conversion has occurred within the fertile mountain valleys and has displaced prairie dogs from central potions of population areas (Longhurst 1944). Before the conversion of lands for agriculture and urbanization, many of these areas likely provided good prairie dog habitat, but now prairie dogs are actively culled on agricultural lands (Knowles 2002; Witmer and Fagerstone 2003). The direct loss of habitat and associated consequences of shooting, poisoning, and habitat fragmentation may lead to large changes in population size and connectivity around these regions (USFWS 2013). Reduced colony size and greater inter-colony distances associated with habitat conversion can increase the likelihood of colonies being extirpated and possibly a localized extinction occurring (Cully 1993; Lomolino et al. 2003).

Most research conducted on prairie dogs, including their movement, has focused on the short and mid-grass prairie dwelling black-tailed prairie dog, *C. ludovicianus* (Roach et al. 2001; Sackett et al. 2012; Pigg 2014). *C. ludovicianus* lives in large colonies on open grassland habitats and individuals have been found to disperse moderate distance from natal colonies (2-4 km on average, but distances up to 10 km kilometers have been observed; Antolin et al. 2006, Garrett and Franklin 1988). Dispersal is also primarily male biased, as males are much more likely to disperse away from natal colonies while females often remain within them (Garrett and Franklin 1988). Certain land types, such as aquatic features, developed lands, and forests are considered poor habitat for prairie dogs and are expected to resist dispersal (Antolin et al. 2006; Sackett et al. 2012). On the shortgrass steppe, evidence has suggested that black-tailed prairie dogs follow drainage

systems while dispersing from natal colonies (Roach et al. 2001). Research on *C. ludovicianus* is likely to be relevant for how *C.g. gunnisoni* moves across the landscape, with biological consideration for the differences between species inhabiting flat open grasslands compared to mountainous environments.

I addressed a series of questions regarding how *C.g. gunnisoni* is distributed across their range: **1)** What landscape features influence *C.g. gunnisoni* dispersal? **2)** Where do connected habitat patches occur and what landscape barriers sperate habitat patches? **3)** Where are habitat corridors that connect *C.g. gunnisoni* colonies located? And **4)** how does habitat conversion alter the paths prairie dogs use to move across the landscape? My goals with this work were to develop a landscape resistance model for estimating how the landscape resists the movement of *C.g. gunnisoni* and to use this model to create flow maps that predict how individuals are likely to move between populations.

The field of landscape genetics (Manuel et al. 2003) incorporates the fields of landscape ecology and population genetics. Landscape genetics aims to identify how the landscape impacts microevolutionary processes including gene flow, selection, and genetic drift (Manel et al. 2003; Holdereggeret al. 2006). To model prairie dog movement, I used circuit theory—implemented via the program Circuitscape (McRae et al. 2008; Hall et al. 2021; www.circuitscape.org). Circuit theory has been useful for generating hypotheses about population connectivity for a wide range of species and scenarios, identifying important habitat corridors, and predicting the effects of changes in landscapes for populations (Batha and Otawa 2013; Dickson et al. 2019, McRae et al. 2016). I utilized Circuitscape for developing a landscape resistance model for *C.g. gunnisoni*. With this resistance model, I can make predictions about dispersal paths used by *C.g. gunnisoni*, estimate the degree of colony connectivity, and identify isolated populations.

2.3 MATERIALS AND METHODS:

Study System:

This study encompasses the range of the *C.g. gunnisoni* subspecies of the Gunnison's prairie dog, as described in Sackett et al. 2014 (Figure 2.1). Gunnison's prairie dogs occupy shrubland and grassland habitats within valleys and mountain meadows in the upper portions of the Rio Grande, South Platte, Arkansas, Gunnison, Uncompahgre, and San Miguel watersheds (Seglund et al. 2005). Most colonies are found in intermountain basins and valleys, including the San Luis Valley (SLV), South Park, Gunnison Basin, Arkansas River Basin, and the Wet Mountain Valley (Seglund et al. 2005; see Figure 2.1 and 2.5). Potential hybrid zones between *C.g. gunnisoni* and *C.g. zuniensis* occur in the southern portion of the range in New Mexico and in the western portion of the San Miguel region (Figure 2.1; Sackett et al. 2014; Joubran 2020). The term "prairie dog" will refer to *C.g. gunnisoni* for the remainder of this chapter unless other species are specified.

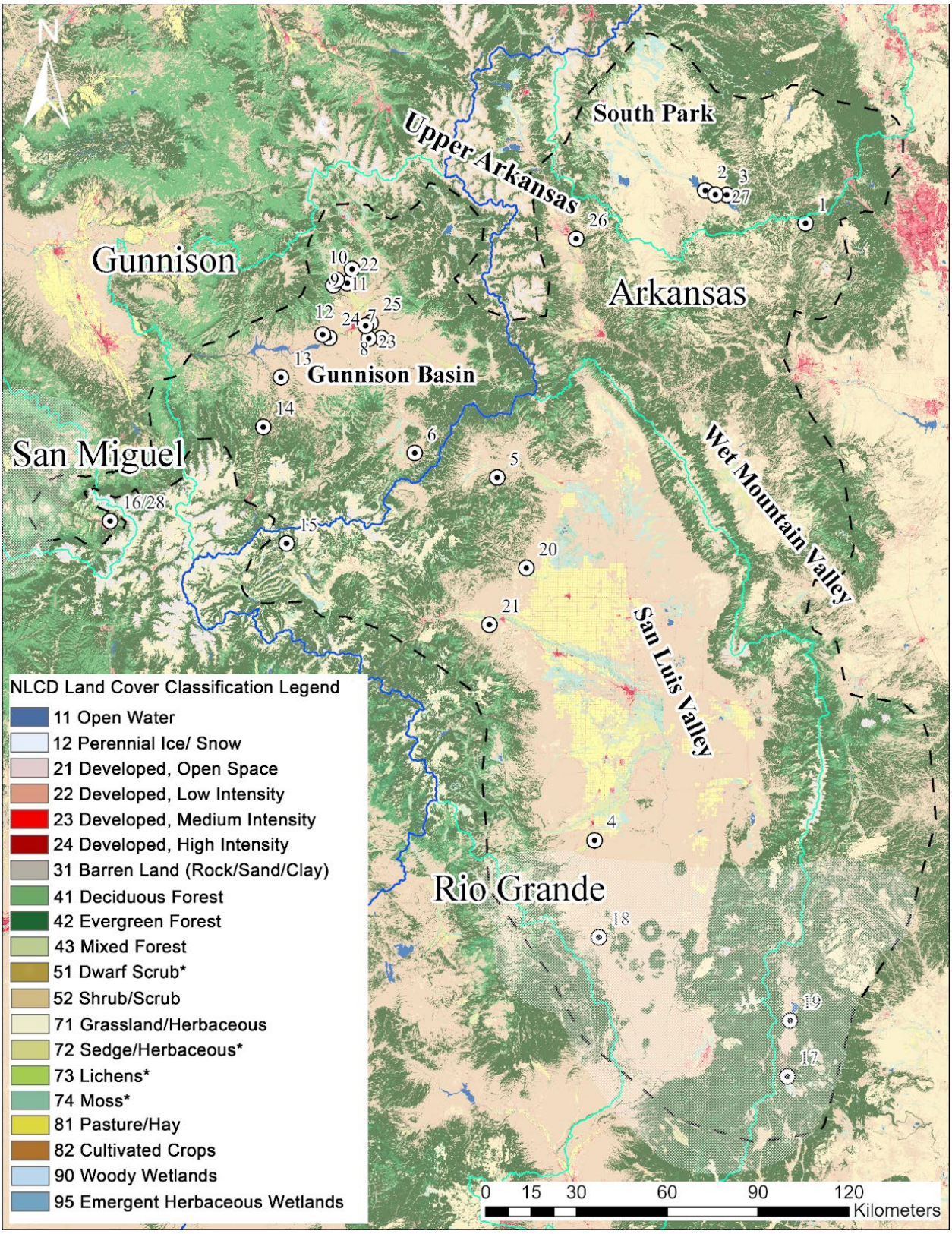


Figure 2.1: The approximate range of *C.g. gunnisoni* is outlined with a black dotted line. All colonies that were sampled are labeled with their unique colony ID (white and black points). The National Land Cover Database (NLCD; USGS) is provided as the background showing the diverse habitat types within the region. The area is dissected by several river drainages, the boundaries of which often follow along high mountain ranges. To the west of the Continental Divide (Blue line running north to south) is the Colorado River Watershed. The Gunnison and San Miguel sub basins (cyan borders) are specific regions within the Colorado watershed that are inhabited by *C.g. gunnisoni*. To the east of the Continental Divide are the Rio Grande, South Platte, and Arkansas Watersheds. *C.g. gunnisoni* inhabit many of the high elevation sub basins within each of these regions. Large parks, valleys, and basins dispersed throughout the area provide good quality prairie dog habitat and is where most colonies are found. Potential hybrid zones between *C.g. gunnisoni* and *C.g. zuniensis* occur in the far west and south of the study area and are shaded white.

Sample collection:

Genetic samples from colonies distributed across the subspecies range were obtained over two sampling periods, resulting in two sets of genetic data (Table 2.1). Microsatellite genotype data is utilized from samples collected during 2008-2010 (See methods from Sackett et al. 2014; Figure 2.1 colonies 17-29). Genomic DNA was collected from individuals which were trapped in 2017 Figure 2.1 (colonies 1-16). Prairie dog trapping and sample collection was conducted in accordance with protocols approved by the University of Colorado's Institutional Animal Care and Use Committee (IACUC #2553). Research followed ASM guidelines (Sikes et al. 2016). Prairie dog trapping methods were adapted from those used in Sackett et al. 2014 and Tripp et al. 2015. At each sampling location, 50-75 Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were distributed on active burrows across each sampled prairie dog colony. Traps were wired open and pre-baited with a corn-oats barley mixture (Ex: 8% 3-way sweet feed: Manna Pro Corp, St. Louis, Missouri, USA, or similar) for at least seven days prior to trapping, allowing prairie dogs to become habituated to the traps. During active trapping events, traps were unwired, set, and baited prior to sunrise. Workers left the site until midmorning or until temperatures reached 23 degrees (C). At this point workers returned, collected captured animals, and closed any open traps. Captured prairie dogs were anesthetized with an isoflurane oxygen

mixture to allow for safe handling. Blood (0.5-1.0 ml) was collected from the femoral vein of each captured prairie dog, stored in a sodium dodecyl sulfate (SDS) solution, and kept cool until placed in a -20C freezer. After prairie dogs recovered from anesthesia, each was returned to the capture location and released.

Table 2.1: Population ID and genetic diversity information for each population utilized from SNP and microsatellite (M-sat) datasets. Sample size (# of individuals) and observed and expected heterozygosity (H_O & H_E) are reported. The approximate elevation (meters above mean sea level) of the colony is given.

Population ID	Data	# of Individuals	H_O	H_E	Elevation (m)
1	SNP	3	0.28	0.3	2672
2	SNP	11	0.32	0.32	2641
3	SNP	13	0.27	0.29	2615
4	SNP	10	0.28	0.29	2427
5	SNP	11	0.28	0.27	2674
6	SNP	14	0.28	0.28	2817
7	SNP	4	0.26	0.31	2432
8	SNP	12	0.28	0.27	2392
9	SNP	6	0.28	0.26	2895
10	SNP	13	0.28	0.28	2502
11	SNP	9	0.27	0.29	2647
12	SNP	8	0.3	0.3	2511
13	SNP	11	0.27	0.28	2556
14	SNP	13	0.28	0.27	2503
15	SNP	8	0.29	0.29	3132
16	SNP	11	0.4	0.31	2663

Table 2.1. Continued

17	M-sat	27	0.41	0.46	2438
18	M-sat	22	0.58	0.56	2406
19	M-sat	21	0.48	0.51	2627
20	M-sat	27	0.62	0.59	2401
21	M-sat	24	0.61	0.6	2614
22	M-sat	41	0.53	0.54	2508
23	M-sat	19	0.51	0.54	2489
24	M-sat	11	0.37	0.37	2352
25	M-sat	6	0.55	0.58	2304
26	M-sat	29	0.58	0.58	2389
27	M-sat	15	0.37	0.32	2503
28	M-sat	18	0.16	0.18	2663

Molecular Methods:

Whole genomic DNA was extracted from blood samples using phenol-chloroform DNA extraction methods (ThermoFisher Scientific) or Qiagen DNeasy Blood & Tissue Kits. Two types of genotype data were used: microsatellite repeats (see Sackett et al. 2014) and whole genome shotgun sequences. Nextera XT DNA libraries were created by the BioFrontiers Institute Next-Gen Sequencing Core Facility and were used to obtain DNA sequences for 192 samples. Illumina Hi-seq platforms using 150 bp paired-end reads were used to produce whole genome sequence data (sequenced by Novogene). To improve genotyping accuracy, a self-similarity filter, according to Lynch et al. 2016, was run on the Gunnison's prairie dog reference genome (Genome assembly ASM1131664v1; Tsuchiya et al. 2020). This filtered out regions of contigs greater than 500 bp in length with greater than 97% identity. Genotypes were called using BWA-MEM to identify variants and produce a Variant Call Format (VCF) (Danecek et al. 2011). To reduce the amount of missing genotype data, individuals with sequencing coverage less than the prairie dog genome size (2.4gb; Tsuchiya et al. 2020) were removed from the analysis. The resulting VCF was filtered to max-missing of 0.5, a minor allele frequency of 0.05, and a minimum quality score of 30.

Microsatellite genotype data came from Sackett et al. 2014 and consist of 16 loci (Jones et al. 2005; Sackett et al. 2009). Additional filtering of microsatellite data led to removing individuals with less than five loci and populations with less than four sampled individuals. Measures of allelic fixation between each pair of populations were obtained by calculating F_{ST} using VCFtools for SNP data, while Arlequin was used for microsatellite data (Wright 1965; Excoffier et al. 2010; Danecek et al. 2011). I use genetic distance, Slatkin's linearized F_{ST} (F_{ST} / (1- F_{ST}); Slatkin 1995), as the measure of genetic differentiation and the response variable.

Landscape Genetics:

To evaluate if isolation-by-distance (IBD) is absent in *C.g. gunnisoni*, I calculated the log transformed geographic distances between sampled populations in ArcGIS Pro (V2.5.1 ESRI) (Slatkin 1993). To test the assumption of IBD in genetic mutation–migration–drift equilibrium I used a Mantel test to find the correlation coefficient between a genetic distance and geographic distance (Guillot and Rousset 2013). When the Mantel statistic is high (r approaches 1), it will indicate that there is strong support that genetic distance increases as geographic distance increases. Mantel tests have been criticized in landscape genetic applications, but these tests can still be valuable and powerful for analyzing multivariate data between pairwise distances (Legendre and Fortin 2010; Diniz-Filho et al. 2013)

Isolation-by-resistance (IBR) methods require the estimation of measures that describe how landscape influences movement or gene flow. I assigned values of resistance (i.e., how difficult it is to move between each pair of populations) that correspond to the landscape features within that space. These resistance values were used to develop a resistance matrix that models the landscape as a resistance surface. Resistance was estimated between each pair of colonies using random walk simulations in the program Circuitscape (McRae 2006; McRae and Beier 2007; McRae et al. 2008). Circuitscape implements a circuit theory approach to calculate the resistance of one amp of electric current moving between two nodes located on an electric circuit. Each population on the landscape is represented as a node and current flows from one node to another through all possible paths across the matrix, with each cell representing a resistor. Each cell will correspond to an area on the landscape, and the value within the cell represents the resistance

encountered to move through that area of the landscape. Geospatial and landscape information was obtained through open-source data from the United States Geological Survey (USGS) and utilized in ArcGIS Pro (V2.5.1 ESRI). The reclassify function was used in ArcGIS Pro to provide resistance values for landscape attributes. The resulting resistance matrices were exported as ascii files and to be used in landscape resistance modeling in the program Circuitscape.

Land class resistance surfaces were developed using the National Land Cover Database (NLCD 2016, USGS, 30X30m; Dewitz 2019) raster surface (Figure 2.1). The sixteen NLCD land classes included within the boundaries of the study system were collapsed into eight different categories expected to be biologically relevant for prairie dogs (Table 2.2) (methods adopted from Sackett et al. 2012). To test how land class influences connectivity, a resistance surface was created for each of the eight biological land classes. For each surface, the land class that was tested was assigned a high resistance value, while all other land types were assigned low resistance values. The resistance between each pair of sampled colonies was calculated by performing separate runs in Circuitscape using one of the eight land class resistance surfaces in each run. Partial Mantel tests (Smouse et al. 1986) were performed between the resulting pairwise resistance values and pairwise genetic differentiation as the response variable, while accounting for geographic distance. Positive correlation coefficients with low p-values indicate that the resistance and genetic distance values show significant correlation and there is evidence that the landscape feature being tested likely resists the movement of Gunnison's prairie dogs. Negative correlation values with low p-values indicate a significant negative relationship, suggesting the landscape feature may be conductive, facilitating dispersal. Each landscape resistance model was evaluated for how well it explains genetic differentiation (Mantel statistic (r)) and if the result is not expected to occur due to random chance, low p-value.

Table 2.2: Land classification types from the USGS National Land Class Database (2016) are listed. These classifications were combined into "functional land types", biologically relevant for prairie dogs. The percentage of each land type that makes up the study area is given.

NLCD land class		Functional land types	Percent of area covered
11	Open water	Water/ice	0.33
12	Perennial ice/snow		
21	Developed, open space	Developed land	1.68
22	Developed, low intensity		
23	Developed, medium intensity		
24	Developed, high intensity		
31	Barren land	Barren land	1.21
41	Deciduous forest	Forest	41.12
42	Evergreen forest		
43	Mixed forest		
52	Shrub/scrub	Shrub/scrub	37.41
71	Grasslands/herbaceous	Grasslands/herbaceous	13.72
81	Pasture/hay	Planted/cultivated	8
82	Cultivated crops		
90	Woody wetlands	Wetlands	1.94
95	Emergent herbaceous/ Wetlands		

A digital elevation model (⅓ arc-second DEM) from the USGS was used in ArcGIS Pro (ESRI) for estimating the dependence of resistance on elevation and slope. The average slope of the landscape in each cell (approximately 10X10m; slope in degrees) was translated as a resistance score ranging from a theoretical minimum integer value of 1 (0–1-degree slope) to a possible maximum of 90 (vertical terrain). To provide biological boundaries to species range limits, I incorporated an effect of resistance on elevational ranges outside of the habitable range. An "elevation" resistance surface was created that assigned elevations above and below the inhabited

range of *C.g. gunnisoni* as resistant to movement (resistance = 100) and regions within the elevational range as low resistance (resistance = 1). These elevations were buffered to be at least 100 meters above and below the highest and lowest known *C.g. gunnisoni* colonies. All resistance surfaces were rasterized at a resolution of 50X50m per cell to provide moderately high resolution to a highly heterogeneous landscape, while reducing raster sizes and thus computation time in Circuitscape.

Developing a landscape resistance surface:

I used two different tests for evaluating how well isolation-by-resistance explained genetic differentiation among sampled localities: Mantel tests and a mixed model approach with maximum likelihood population effects models (MLPE's). Each landscape feature was evaluated independently with simple and partial Mantel tests and together with MLPE models. Mantel tests were used to evaluate the effect that geographic distance (log transformed) has on genetic distance. Distance is to an extent incorporated in each resistance model as resistance increases with greater geographic distance. Simple and partial Mantel tests were performed to evaluate IBR for each model and partial Mantels would account for the effect of geographic distance (9999 permutations using the R package "vegan V2.5-6"; Oksanen 2013). Mantel tests were conducted separately for SNP and microsatellite data. The correlation coefficient was also calculated between values of genetic differentiation from both markers.

The lme4 R package (Bates et al. 2015) was used to implement Maximum Likelihood Population Effects models (MLPE; Clarke, et al. 2002; Van Strien et al. 2012; Row et al. 2017) using genetic differentiation as the response variable. MLPE is a linear mixed effects modelling technique that models landscape resistance as a fixed effect and pairwise comparisons as a random effect term that accounts for nonindependence in pairwise data (Balkenhol et al. 2016; Shirk et al.

2018; Trumbo et al. 2019). The explanatory variables included the log of geographic distance and pairwise resistance values from "elevation", "land class", and "slope" models. All predictor and response variables were standardized around zero using a z-scoring method (R datawizard package). To account for non-independence in pairwise data, colony pairs were included as a random effect term in each MLPE model.

Multiple methods are commonly used to evaluate MLPE models. Reporting AIC, BIC, marginal or conditional R^2 have been widely used (Row et al. 2017). Bayesian information criterion (BIC, obtained from each fitted MLPE model using the Dredge function in the MuMIn R package; Barton and Barton 2015) was used to order which resistance best explains genetic differentiation (Shirk et al. 2017). I also used the rsquared() function in the R package PIECEWISESEM (Lefcheck, 2016), to calculate marginal R^2, a measure that includes fixed effects and conditional R^2 a measure that includes both fixed and random effects (Nakagawa and Schielzeth 2013). Delta BIC, marginal R^2, and conditional R^2 for microsatellite, SNP data, and combined datasets were calculated. If any of the landscape features; Elevation, Land class, Slope, or Geographic Distance, were omitted from the top models, then there would be evidence to not include the feature in a finalized model.

Each landscape feature implicated as a source of resistance to prairie dog movement was incorporated into a single landscape resistance surface that described how the landscape influences population connectivity of *C.g. gunnisoni.* The "Raster Calculator" function in ArcGIS Pro was used to create a single raster by summing each individual resistance surface, then scaling resistance measures as integers between 1 as the lowest resistance value and 100 as the highest resistance value. These methods simplified the process of incorporating multiple landscape features into a single model. It is likely that each feature contributes a different amount of resistance to prairie

dog movement, but model optimization, such as a method used in the ResistanceGA R package (Petterman 2018) is computationally expensive and would be unfeasible to use on the large datasets in this study. The cumulative resistance model was run in Circuitscape to calculate resistances between each pair of populations. Mantel, partial Mantel, and Pearson correlations were used to test the correlation between the measured pairwise resistances from the final model and genetic differentiation. A cumulative flow model was also produced that shows where on the landscape movement between the 28 sampled colonies is expected to occur.

Current flow maps that are produced by Circuitscape estimate the amount of current that flows between the population locations in the model, and thus each map is subjected to interpretation based on the populations that are used. Populations need to be carefully selected in order to develop a current map for a desired outcome. The colonies that were included in this analysis spanned much of the occupied range, but many areas within the *C.g. gunnisoni* range were unsampled and the sampling effort varied greatly. High intensity sampling occurred in the Gunnison Basin, while lower intensity sampling occurred in the San Luis valley and South Park, and no samples were collected in southeast Colorado. One question I was interested in was understanding how population areas are connected to each other. To evaluate connectivity across the entirety of the subspecies range, I developed a population file that included colonies that were distributed across the entire range. To do this, I first collected information on colony occupancy from the Bureau of Land Management, the US Forest Service, Colorado Parks, and Wildlife, and from personal observations. A 50X50 km grid was overlaid across the range and I then removed all but one colony from each cell. If possible, colonies were kept that increased inter-colony distances but were otherwise randomly chosen. This selection of populations reduces the effect

colony density can have on the resulting cumulative conductance maps and significantly decreases computational time by decreasing the number of pairwise calculations within Circuitscape.

Habitat Conversion:

The effect of regional conversion of prairie dog habitat for agricultural purposes on the predicted connectivity of prairie dogs was evaluated for two focal areas: the San Luis Valley and the Gunnison basin. A - cultivated - resistance surface was created using the same methods as previously described, except those lands described as "cultivated" in the NLCD dataset were incorporated as a high-resistant land class. A connectivity model in Circuitscape was created using the -original resistance surface- for the first run and the -altered cultivated surface- in the second run. To observe changes in expected connectivity, resulting conductance maps were subtracted from each other using the Raster Calculator function in ArcGIS Pro.

2.4 RESULTS:

Study System:

Within the study area, shrubland and grassland habitats, which correspond with the greatest occupancy of prairie dogs, made up nearly half of the lands in the region. Forested habitat accounted for most of the remaining landscape (41% of the area in the region). Barren lands, wetlands, water, urban, and agricultural land types made up less than 10% of the total remaining area in the region (Table 2.2). Elevation of sample sites ranged from a minimum of 2304 to a maximum of 3132 meters above sea level.

Molecular Results:

I analyzed 648,881 SNPs from 156 genotyped prairie dogs sampled across 16 colonies. I also incorporated data from 16 microsatellite alleles from 260 individuals sampled across 12 colonies (see Figure 2.1 for additional details). The allelic differentiation between pairs of colonies (F_{ST}) ranged 0.01 to 0.33 for SNP data and 0.03 to 0.79 for microsatellite data (Tables 2.3 and 2.4). Expected heterozygosity ranged from 0.26 to 0.32 for SNP data and 0.18 to 0.60 for microsatellite data (Table 2.1)

Table 2.3: SNP data; Top Triangle are geographic distances between colonies (km). Bottom Triangle are pairwise F_{ST} values.

ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		34.8	27.2	215.2	132.1	149.4	149.2	145.1	150.6	156.1	157.2	163.7	180.7	191.3	201.4	251.2
2	0.04		7.7	217.6	117.1	129.0	121.3	117.5	119.6	125.3	126.6	135.1	153.2	165.5	180.9	226.0
3	0.05	0.03		217.4	120.4	133.6	127.6	123.7	126.5	132.3	133.5	141.5	159.4	171.4	185.6	231.8
4	0.12	0.14	0.15		124.4	141.2	182.1	180.3	205.1	203.9	202.4	189.9	184.9	175.1	141.5	193.3
5	0.15	0.15	0.17	0.08		27.9	62.3	59.5	83.6	83.8	82.7	74.3	78.4	78.6	72.7	129.9
6	0.15	0.14	0.17	0.1	0.1		40.9	39.5	64.1	62.7	61.2	49.7	51.0	50.9	52.1	104.8
7	0.18	0.17	0.21	0.11	0.11	0.07		4.2	23.4	21.9	20.6	15.3	31.8	45.6	73.1	105.8
8	0.17	0.16	0.19	0.12	0.12	0.07	0.03		24.7	24.3	23.2	19.5	35.7	48.8	74.8	109.2
9	0.21	0.19	0.21	0.16	0.15	0.1	0.09	0.08		6.2	8.2	23.6	42.7	59.8	93.2	116.2
10	0.19	0.17	0.2	0.13	0.12	0.07	0.05	0.06	0.05		2.3	18.6	37.0	54.3	88.6	110.1
11	0.18	0.17	0.2	0.14	0.12	0.08	0.05	0.06	0.05	0.01		16.3	34.8	52.0	86.4	108.0
12	0.19	0.17	0.19	0.13	0.12	0.07	0.07	0.07	0.08	0.06	0.06		19.8	36.3	70.1	94.4
13	0.19	0.16	0.19	0.14	0.13	0.08	0.09	0.08	0.1	0.09	0.08	0.08		17.4	55.0	74.7
14	0.27	0.22	0.25	0.17	0.18	0.14	0.17	0.15	0.17	0.15	0.15	0.14	0.14		39.2	60.6
15	0.22	0.2	0.21	0.15	0.16	0.14	0.17	0.15	0.17	0.16	0.16	0.15	0.15	0.21		60.3
16	0.33	0.31	0.33	0.2	0.27	0.29	0.32	0.31	0.33	0.32	0.31	0.31	0.32	0.32	0.32	

Table 2.4: Microsatellite data; Top Triangle are geographic distances between colonies (km). Bottom Triangle are pairwise F_{ST} values.

ID	17	18	19	20	21	22	23	24	25	26	27	28
17		77.4	18.4	188.7	178.7	299.6	284.3	287.0	284.4	285.2	292.1	289.4
18	0.23		68.9	124.3	109.4	231.4	216.1	217.1	216.5	230.7	248.2	212.1
19	0.03	0.13		173.0	164.2	284.1	268.9	272.1	268.9	267.6	273.8	278.8
20	0.22	0.18	0.19		22.4	111.1	96.0	100.1	95.9	109.9	138.2	138.4
21	0.31	0.26	0.27	0.25		122.1	106.8	108.5	107.1	130.6	160.4	129.9
22	0.4	0.4	0.38	0.24	0.14		15.3	19.0	15.2	77.0	125.1	111.0
23	0.19	0.22	0.18	0.19	0.15	0.25		12.7	1.9	75.3	123.4	106.3
24	0.53	0.53	0.47	0.37	0.24	0.17	0.4		14.6	87.9	136.1	94.4
25	0.27	0.29	0.22	0.12	0.1	0.08	0.07	0.31		73.4	121.6	108.2
26	0.1	0.07	0.06	0.16	0.25	0.35	0.1	0.49	0.22		48.3	180.0
27	0.17	0.35	0.17	0.42	0.36	0.5	0.29	0.67	0.5	0.2		227.2
28	0.56	0.55	0.52	0.34	0.57	0.55	0.55	0.77	0.56	0.53	0.79	

Support for a resistance surface and flow model:

Correlation coefficients from evaluating IBD using Mantel tests resulted in significant ($P < 0.05$) correlation coefficients of $r = 0.57$ for SNP data and $r = 0.19$ for microsatellite data. Geographic distance does explain genetic differentiation between individual colonies (Figure 2.2).

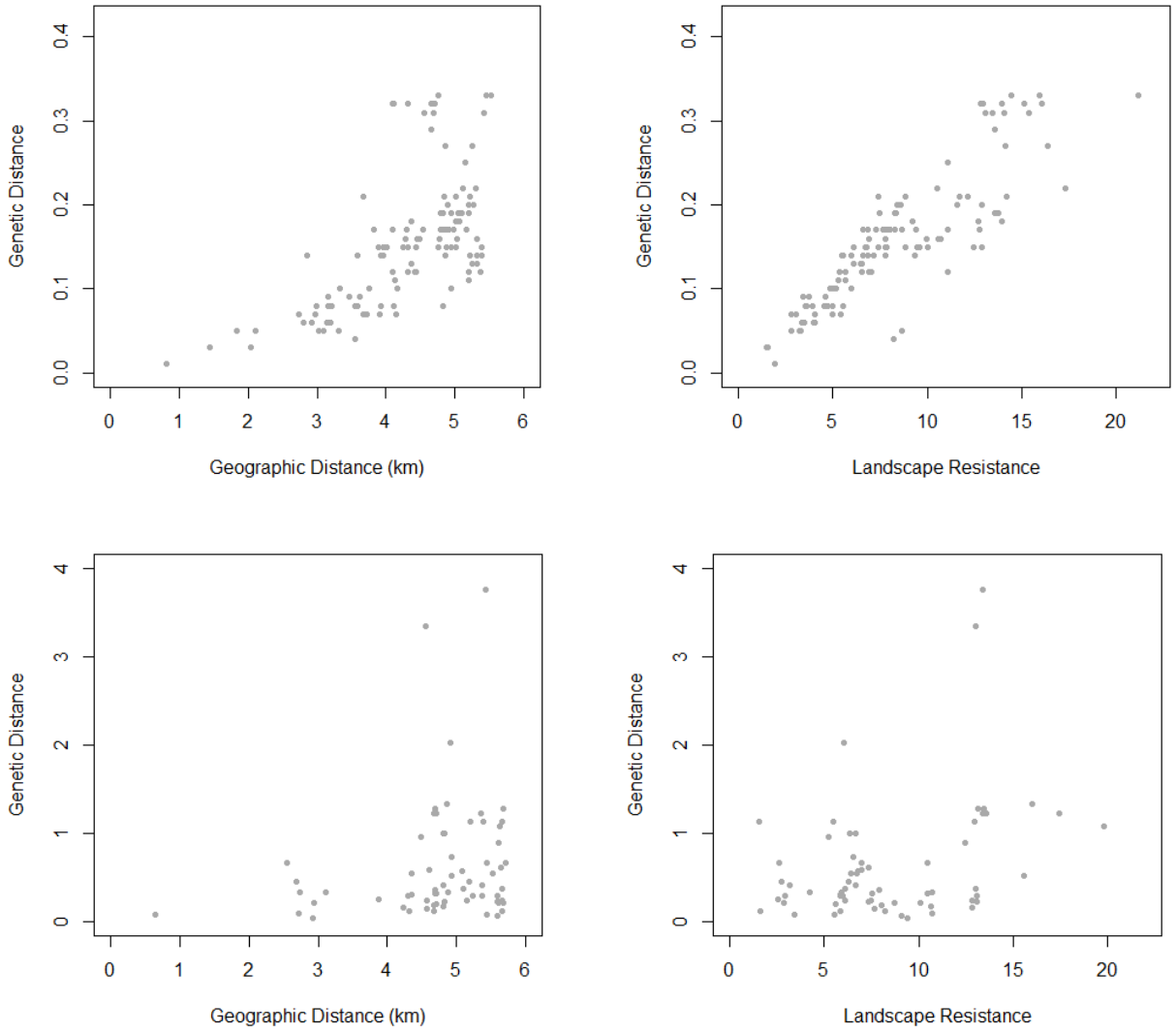


Figure 2.2: Relationship between Genetic distance (Salkin's linearized F_{ST}), and measures of geographic distance (log transformed distance in kilometers) and resistance values calculated from the program Circuitscape. Top plots show data from genetic markers using whole genome data (SNPs) and bottom plots show results using Microsatellite loci (bottom). Mantel Statistic for IBD is $r = 0.57$ (SNP) and 0.19 (microsatellite). Mantel statistics for IBR are $r = 0.78$ (partial Mantel) and 0.85 (Mantel) for SNP data and 0.53 (partial Mantel) and 0.52 (Mantel) for Microsatellite data.

The results of both Mantel and partial Mantel tests that account for geographic distance for each test of IBR are reported in Figure 2.2 and Table 2.6). The strength of the mantel statistic changes slightly, but there does not appear to be a large difference between the two correlation coefficients. Of the eight individual land classes that were tested (Table 2.2), “Water”, “Urban”, and “Forest” models resisted prairie dog movement. For each of the three models, resistance values and genetic differentiation had a correlation coefficient (r) greater than 0.3. Developed and forest models were significant, $p < 0.01$, while water has a significance of $p = 0.055$. The wetlands land class had a lower correlation value of $r = 0.19$ and was less significant ($p = 0.15$) and was therefore not included as a high resistant land class. The correlation coefficients of the other four land classes were poor and non-significant (Table 2.5). Prairie dogs do not live within habitats comprised of forests, water features, and urban environments and each of these three land classes show strong support for resisting the movement of individuals. Each of these three land classes were assigned high resistance values (resistance = 100 in the model) and all other land types were assigned low resistance (resistance = 1) in developing a single land class resistance surface.

Table 2.5: Results of partial Mantel test for individual land classes

Land Class Category	**Partial Mantel (r)**	**p-value**
Water and Snow	0.32	0.055
Developed land	0.67	0.0004
Baren	-0.017	0.512
Forest	0.67	0.0004
Shrubland	-0.281	0.775
Herbaceous	-0.077	0.583
Planted/Cultivated	-0.314	0.913
Wetlands	0.19	0.15

Partial Mantel tests from the land class model resulted in a high correlation with SNP data, but a lower correlation with microsatellite data. Tests on slope and elevation had correlation coefficients between 0.51 and 0.72 for both SNP and microsatellite datasets. Goodness-of-fit measures obtained from combination of both data sets have correlations coefficients between 0.43 and 0.62 for all land class features that were tested (Table 2.6) for additional details on correlation results).

Table 2.6: Correlations between pairwise linearized F_{ST} (both SNP and microsatellite datasets) and pairwise resistance values obtained through Circuitscape using different landscape models. For microsatellite and SNP genotype data, a Mantel test is performed for tests of IBD, while Mantel and partial Mantel tests are used to test IBR while accounting for geographic distance. The r value for the Pearson's correlation coefficient from a combined dataset is given in the far-right column. (p-value < 0.05 are **bold** for Mantel tests)

Landscape attribute	Test	SNP		Microsatellite		Combined genotypes
		Partial Mantel (r)	Mantel (r)	Partial Mantel (r)	Mantel (r)	Pearson's Correlation (Pearson's r)
Geographic distance	IBD	**-**	**0.57**	-	**0.19**	0.43
Elevation	IBR	0.53	**0.67**	**0.64**	**0.53**	0.62
Slope	IBR	**0.63**	**0.72**	**0.51**	**0.53**	0.59
Land class	IBR	**0.72**	**0.80**	0.21	0.27	0.61
Combined:	IBR	**0.78**	**0.85**	**0.53**	**0.52**	0.67

There were multiple statistically plausible models supported by MLPE models with variation in the top models occurring between BIC, marginal R^2 and conditional R^2 among the SNP, microsatellite, and combined datasets. Each of the landscape attributes—geographic distance, land types, slope, and elevation— are included in a top model among the three datasets and occur multiple times in the top 5 models using BIC, marginal R^2 and conditional R^2 methods. Each landscape features shows some predictive power based on Mantel and MLPE tests for influencing population connectivity; therefore, I did not find evidence to exclude any of the landscape features from being used to create a "finalized" resistance model.

Table 2.7: Maximum Likelihood population effects model results accounting for each pair of populations as a random effect. Models are included for SNP and microsatellite data, SNP data only and microsatellite data only. Landscape features included elevation, land class, slope and the log of geographic distance. Multiple model rankings are included: Delta BIC, marginal R^2 (R^2m), and conditional R^2 (R^2c). Colors scale range from supported models (green) to unsupported models (red). Models that have a Delta BIC within 5 of the top model are **bolded.** Models within 0.03 of the top model are *italicized* for Marginal R^2 (R^2m) and underlined for conditional R^2 (R^2c).

Model #	Dataset	Elevation	Land Class	Slope	Distance	Delta BIC	R^2m	R^2c
1	SNP+Micro					36.24	0.00	0.82
2	**SNP+Micro**	**0.41**				**4.06**	**0.21**	**0.77**
3	*SNP+Micro*		*0.60*			*6.98*	*0.35*	*0.84*
4	**SNP+Micro**	**0.27**	**0.28**			**4.31**	**0.30**	**0.79**
5	**SNP+Micro**			**0.46**		**0.00**	**0.26**	**0.79**
6	**SNP+Micro**	**0.20**		**0.29**		**1.74**	**0.26**	**0.76**
7	***SNP+Micro***		***0.22***	***0.34***		***3.19***	***0.32***	***0.80***
8	SNP+Micro	0.17	0.14	0.24		6.10	0.30	0.78
9	SNP+Micro				0.25	15.31	0.07	0.81
10	SNP+Micro	0.42			-0.01	9.28	0.22	0.77
11	SNP+Micro		0.45		0.09	10.36	0.28	0.82
12	*SNP+Micro*	*0.33*	*0.30*		*-0.06*	*9.04*	*0.33*	*0.79*
13	SNP+Micro			0.44	0.02	5.13	0.25	0.78
14	SNP+Micro	0.27		0.32	-0.08	6.15	0.30	0.77
15	*SNP+Micro*		*0.22*	*0.35*	*-0.01*	*8.39*	*0.33*	*0.80*
16	*SNP+Micro*	*0.25*	*0.17*	*0.26*	*-0.10*	*10.13*	*0.35*	*0.79*
1	SNP					109.46	0.00	0.92
2	SNP	0.75				29.83	0.57	0.95
3	***SNP***		***1.26***			***3.57***	***0.79***	***0.98***
4	*SNP*	*0.15*	*1.10*			*5.44*	*0.80*	*0.98*
5	SNP			0.75		37.78	0.54	0.95
6	SNP	0.51		0.30		22.43	0.61	0.95
7	***SNP***		***1.06***	***0.19***		***0.00***	***0.79***	***0.98***
8	***SNP***	***0.00***	***1.06***	***0.19***		***4.79***	***0.79***	***0.98***
9	SNP				0.40	43.89	0.20	0.94
10	SNP	0.52			0.17	26.28	0.51	0.95
11	***SNP***		***1.12***		***0.12***	***1.87***	***0.78***	***0.98***
12	*SNP*	*0.03*	*1.10*		*0.11*	*6.52*	*0.79*	*0.98*
13	SNP			0.42	0.21	34.82	0.42	0.93
14	SNP	0.43		0.24	0.10	24.56	0.57	0.94
15	*SNP*		*1.04*	*0.14*	*0.06*	*3.14*	*0.79*	*0.98*
16	*SNP*	*-0.04*	*1.06*	*0.16*	*0.08*	*7.61*	*0.78*	*0.98*

Table 2.7 continued.

1	Micro					12.92	0.00	0.68
2	Micro	0.33				8.76	0.11	0.60
3	Micro		0.31			13.76	0.08	0.69
4	Micro	0.37	-0.07			12.86	0.10	0.61
5	Micro			0.40		8.84	0.15	0.61
6	Micro	0.19		0.22		12.14	0.13	0.60
7	Micro		-0.09	0.46		12.86	0.14	0.62
8	Micro	0.24	-0.16	0.29		15.77	0.13	0.59
9	Micro				0.09	16.09	0.01	0.68
10	***Micro***	***0.93***			***-0.61***	***0.00***	***0.39***	***0.65***
11	Micro		0.37		-0.05	17.82	0.10	0.70
12	**Micro**	**0.94**	**-0.01**		**-0.61**	**4.20**	**0.38**	**0.65**
13	Micro			0.68	-0.29	8.36	0.30	0.68
14	***Micro***	***0.74***		***0.26***	***-0.60***	***2.16***	***0.42***	***0.65***
15	Micro		0.01	0.67	-0.29	12.56	0.29	0.69
16	*Micro*	*0.77*	*-0.11*	*0.30*	*-0.59*	*5.97*	*0.42*	*0.66*

The final landscape resistance surface (now referred to as the "resistance surface"), combined the individual resistance surfaces of land class, slope, and elevation, into a single model. Distance was partially incorporated in our model as resistance between populations increases with increasing distance (McRae et al. 2008). Pairwise resistance values between sampled colonies were calculated by running Circuitscape with the resistance surface and population file. The correlation coefficients (r) between pairwise resistance values and the genetic differences from partial Mantel tests were 0.78 and 0.57 for SNP and microsatellite data, respectively. The Pearson's correlation coefficient of r = 0.67 resulted from a test that utilized both SNP and microsatellite information (Table 2.6). The cumulative flow model (Figure 2.3) shows the expected connectivity between colonies sampled in this study.

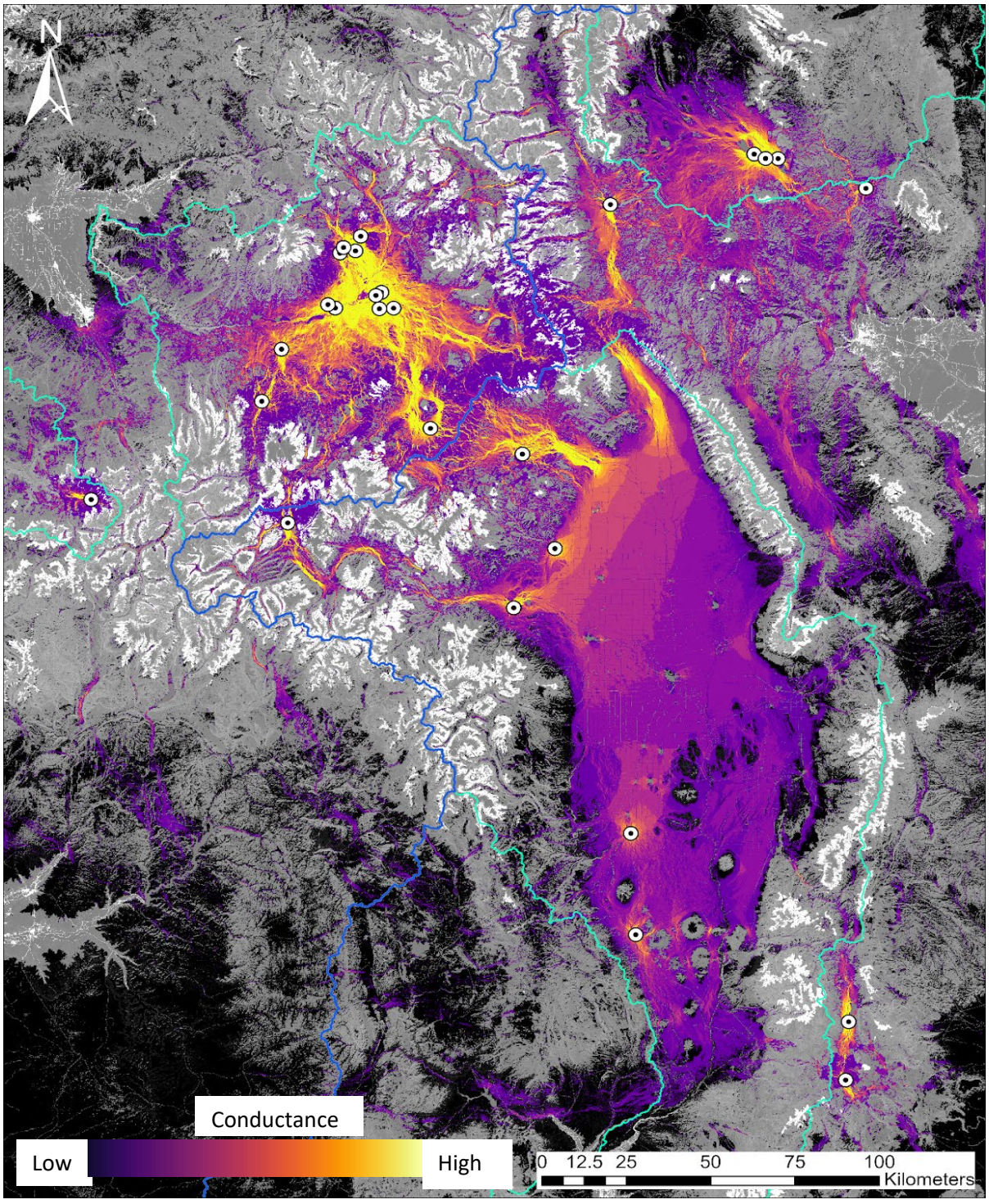


Figure 2.3: A cumulative flow map shows the summation of predicted connectivity between sampled populations (white and black points) laid on top of the final landscape resistance surface ranging from low resistance (dark) to high resistance (light). Conductance values range from low current (dark purple) to higher current paths (yellow). Areas with little to no current were made transparent and allow visualization of the landscape surface.

General features of the occupancy and flow model:

I used a set of colonies located across the entirety of the subspecies range for developing a range-wide flow model (Figure 2.4). I selected the colonies that would be included in the model to be located across the known range of *C.g. gunnisoni* and to be distributed as evenly as possible across the landscape in order to reduce the effect high population density has on Circuitscape's cumulative flow maps. The resulting flow model shows most colonies are connected by multiple conductance paths that vary in width depending on locality. There are two localities that remain unconnected (isolated): one occupied a montane meadow within a narrow canyon located in the upper San Miguel watershed near the mountain town of Telluride, CO (colonies 16 and 27 in the west) and the other consisted of two colonies sampled in a small intermountain valley (colonies 17 and 19) in the southeast portion of the range near the town of Angel Fire, NM. These two areas are disconnected from other colonies in the conductance map, an inference stemming from the observation that these areas are geographically separated from other colonies and are surrounded by resistant (low conductance) landscapes. Each of these isolated colonies is genetically divergent from most other colonies (Tables 2.3 and 2.4).

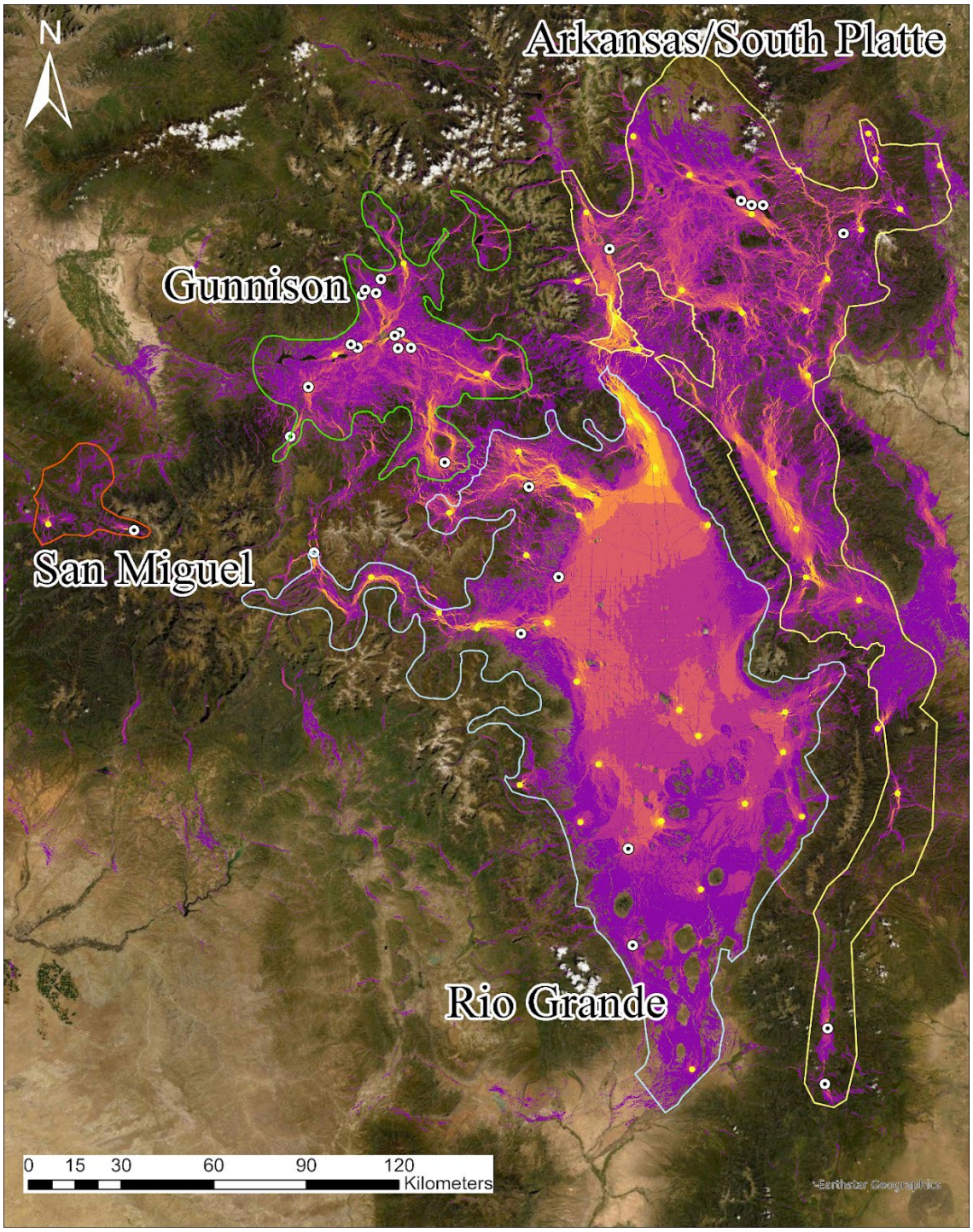


Figure 2.4. Range wide conductivity map based on distribution nodes using the best resistance surface (see Figure 2). This map estimates potential connectivity between populations distributed across the occupied range. Yellow points indicate where populations that were used in the model are. For reference, white dots show where the sampled populations are (see Figure 2.1 for reference). The four population areas expected to encompass the *C.g. gunnisoni* range are outlined. San Miguel (red), Gunnison (green) Rio Grande (light blue), and Arkansas/South Platte (yellow) show the regions that are expected to constitute individual populations.

The resulting cumulative flow model revealed large areas of continuous conductive habitat throughout the species' range. There were three large core areas of high connectivity delimited by the watershed boundaries of the 1) Gunnison, 2) Rio Grande, and 3) the combination of the South Platte and Arkansas River watersheds (Figure 2.4). In addition, there was a small area comprising a fourth core region located in a high elevation portion of the upper portion of the San Miguel watershed. Each of these four areas were separated from each other by resistant landscape features (mostly high elevation).

Hypothesized corridors between population areas:

Using the range-wide flow model, I was able to identify habitat corridors that may or may have facilitated dispersal between population areas. One hypothesized connection evident in the model connects the southeast Gunnison and northwest Rio Grande watersheds through the Cochetopa Hills (Figure 2.5a). Another putative corridor connects the northern end of the San Luis Valley and the Arkansas River Valley through a narrow pass (Figure 2.5b). These corridors may provide interpopulation connectivity between areas that were mostly bordered by the Sangre De Cristo, San Juan, and Collegiate Mountain ranges that form barriers to prairie dog movement.

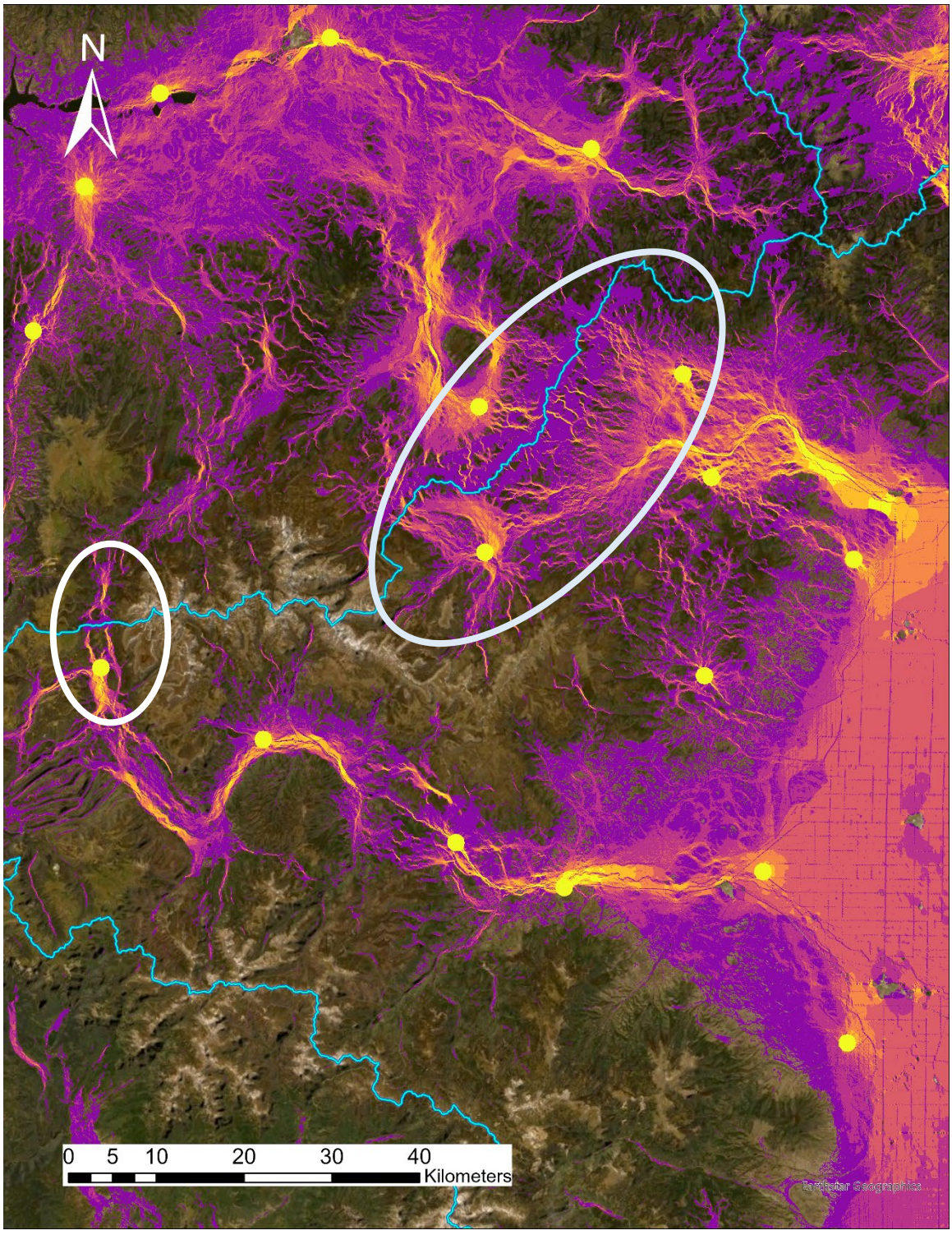


Figure 2.5a: The border between the Gunnison and Rio Grande watersheds. The Cochetopa hills (circled) provide a semipermeable barrier for gene flow to occur across the San Juan mountains and Continental Divide.

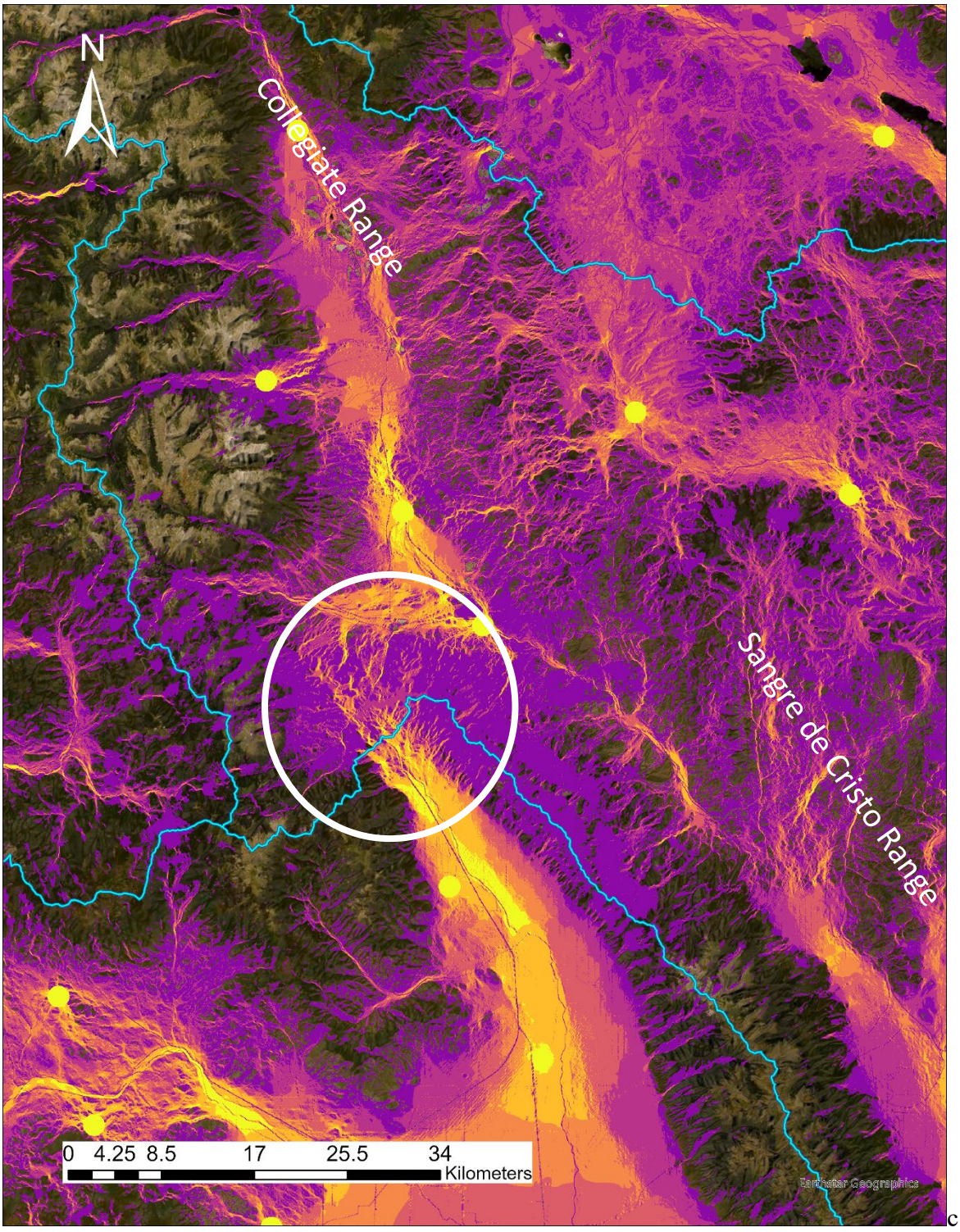


Figure 2.5b: The border between the Gunnison, Arkansas and Rio Grande watersheds. Poncha Pass (circled) provides connectivity between the San Luis Valley (south) and Arkansas river valley (north). The Sangre de Cristo and Collegiate Range are strong barriers separating prairie dog populations.

The effect of cultivated lands on dispersal paths:

The proportion of cultivated lands accounted for approximately eight percent of the lands in the study area (Table 2.1). This small percentage may be of high importance however, because of its centrality in the distribution of putatively suitable habitat (Figure 2.1). The range-wide connectivity model indicated that regions located in the central portions of the Gunnison Basin (Figure 2.6a) and the San Luis Valley (Figure 2.6b) and were expected to provide high landscape connectivity (left map in each Figure 2.6a and 2.6b). When agriculture was assumed to be a resistant land class, these same areas became low conductive areas (right map in each Figure 2.6a and 2.6b). This change in expected landscape connectivity resulted in reductions of the core of each population area and shifted the connectivity to the landscape that surrounds these agricultural zones (Figure 2.6c). In the Gunnison Basin, connectivity decreased in the central portion of the basin along waterways to the north and east. This decrease of connectivity in the central portion of the Gunnison Basin could lead to decreased gene flow across the valley, and the basin could be fragmented into multiple clusters of colonies. In the San Luis Valley (Figure 2.6c bottom), connectivity paths are expected to increase in the western portion of the valley as the central portions of the San Luis Valley becomes more resistant (Figure 2.6d).

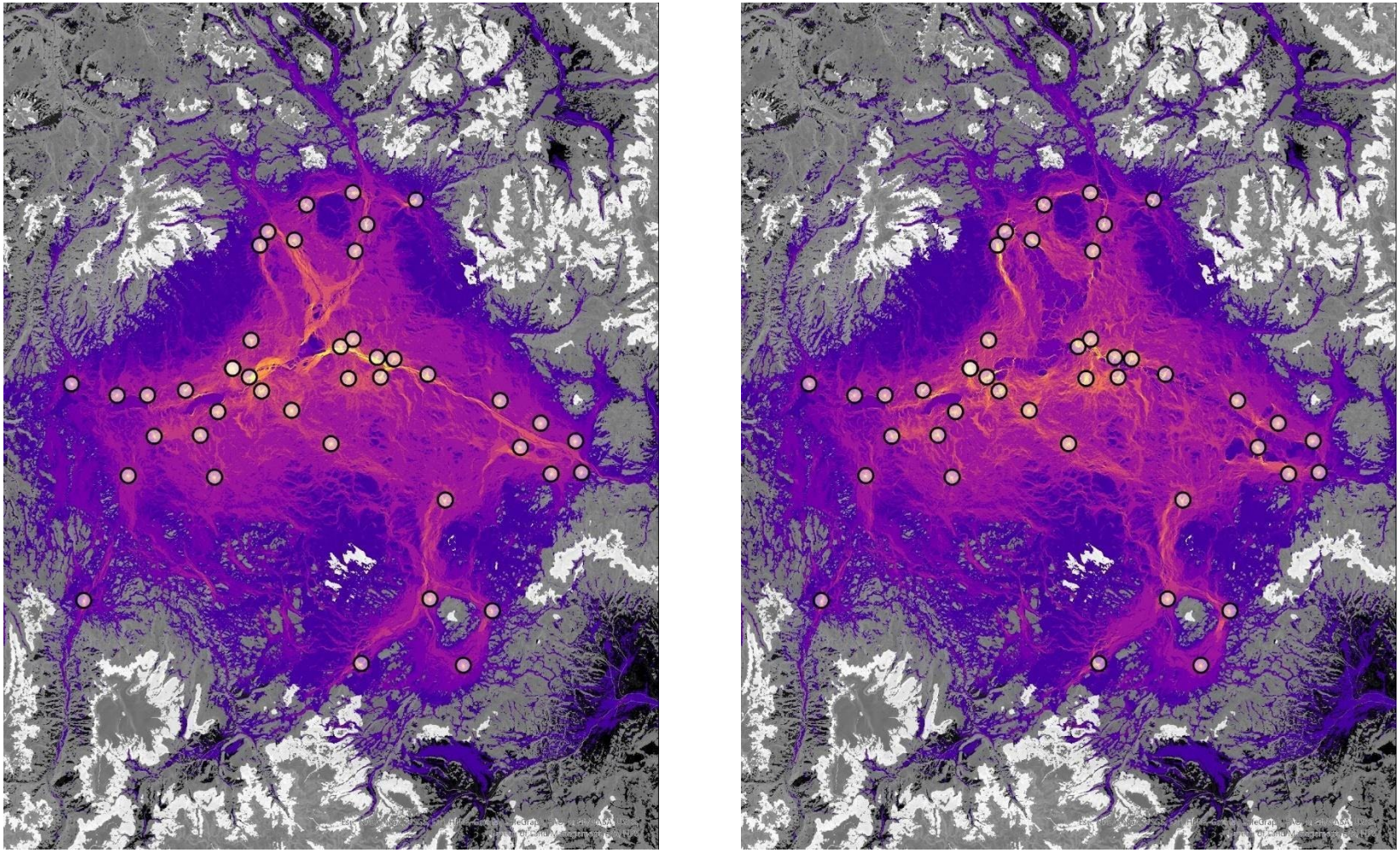


Figure 2.6a: The Gunnison Basin. The map on the left shows a flow model using the finalized resistance surface and a subset of colonies distributed around the region. The map on the right uses the altered resistance surface that includes cultivated lands as a resistance surface.

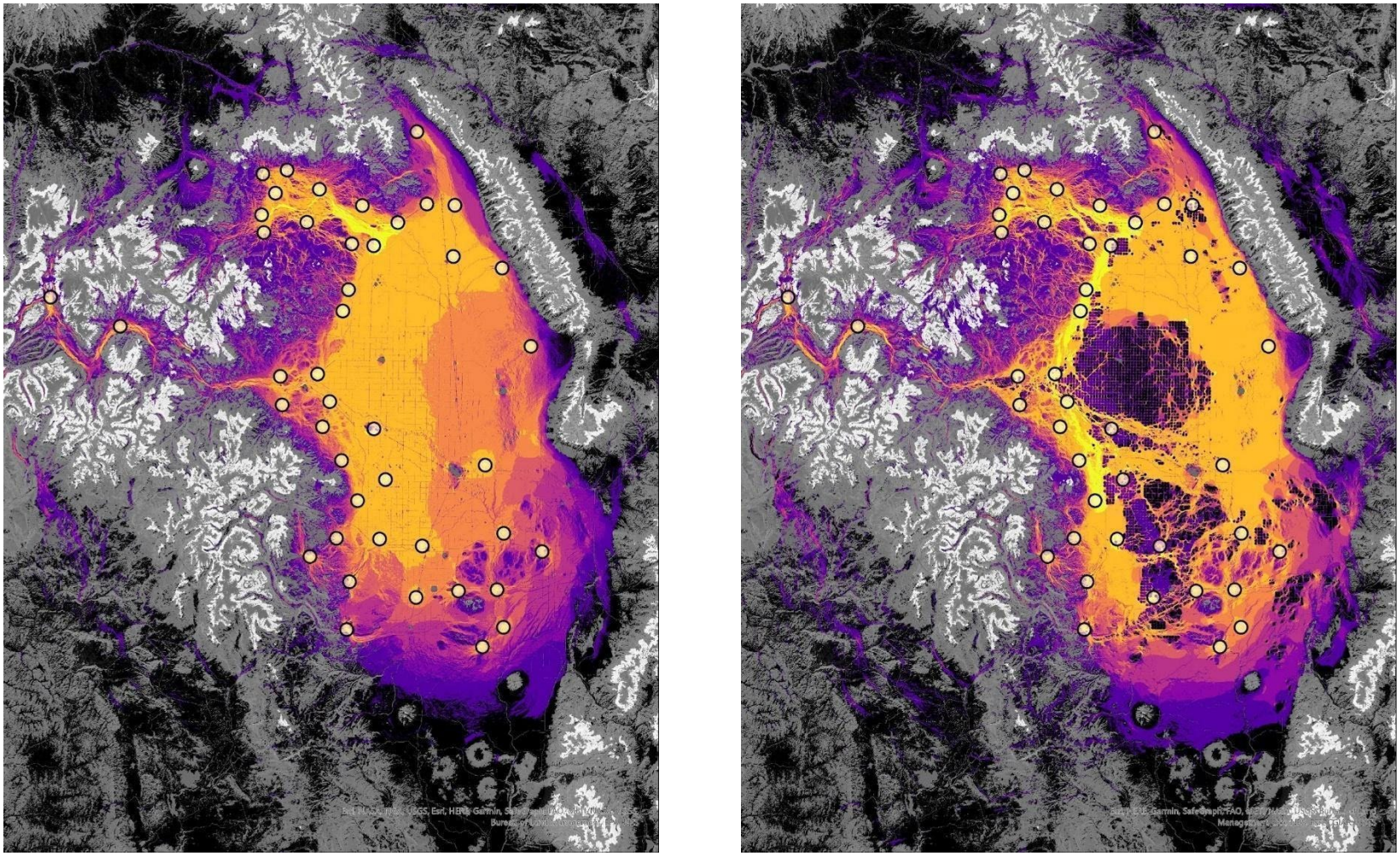
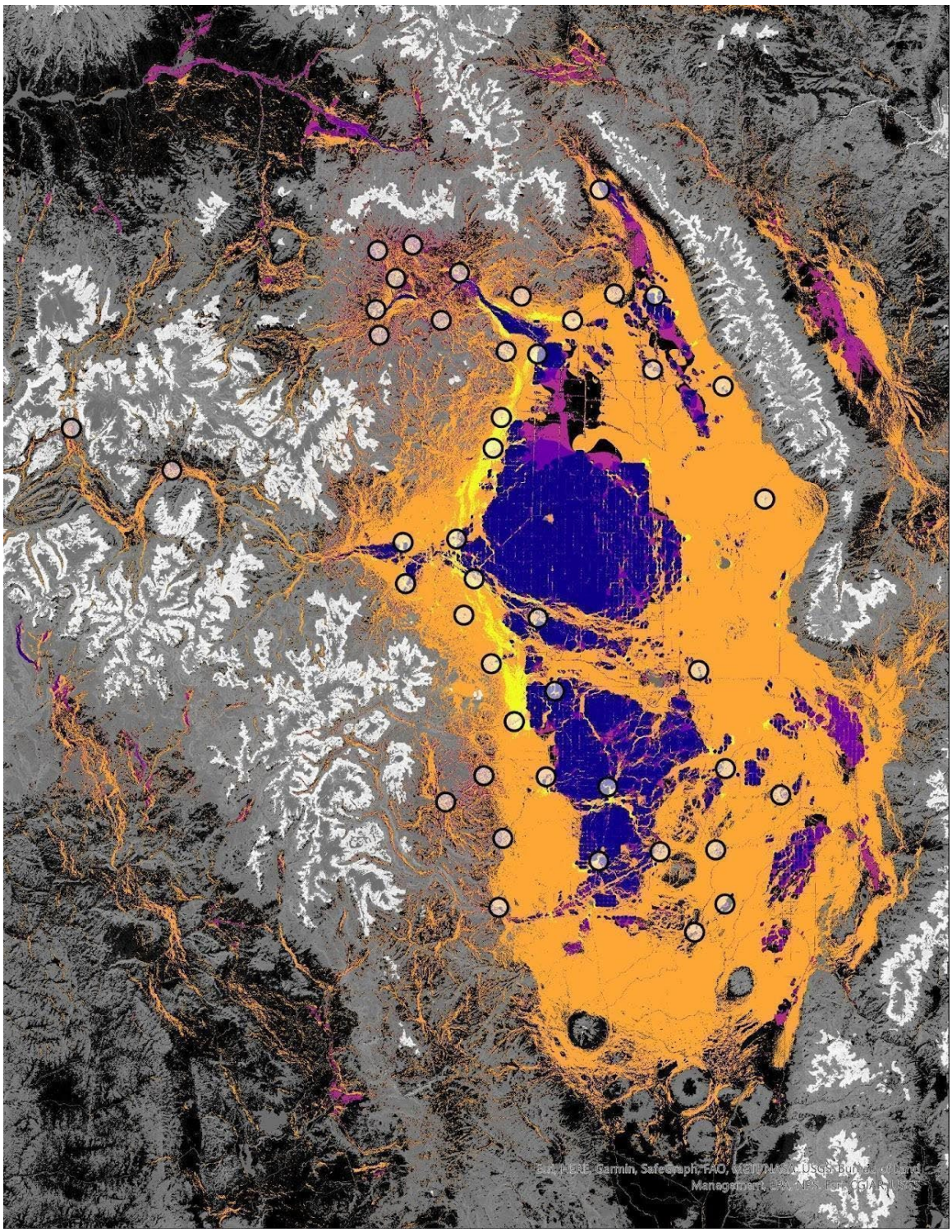
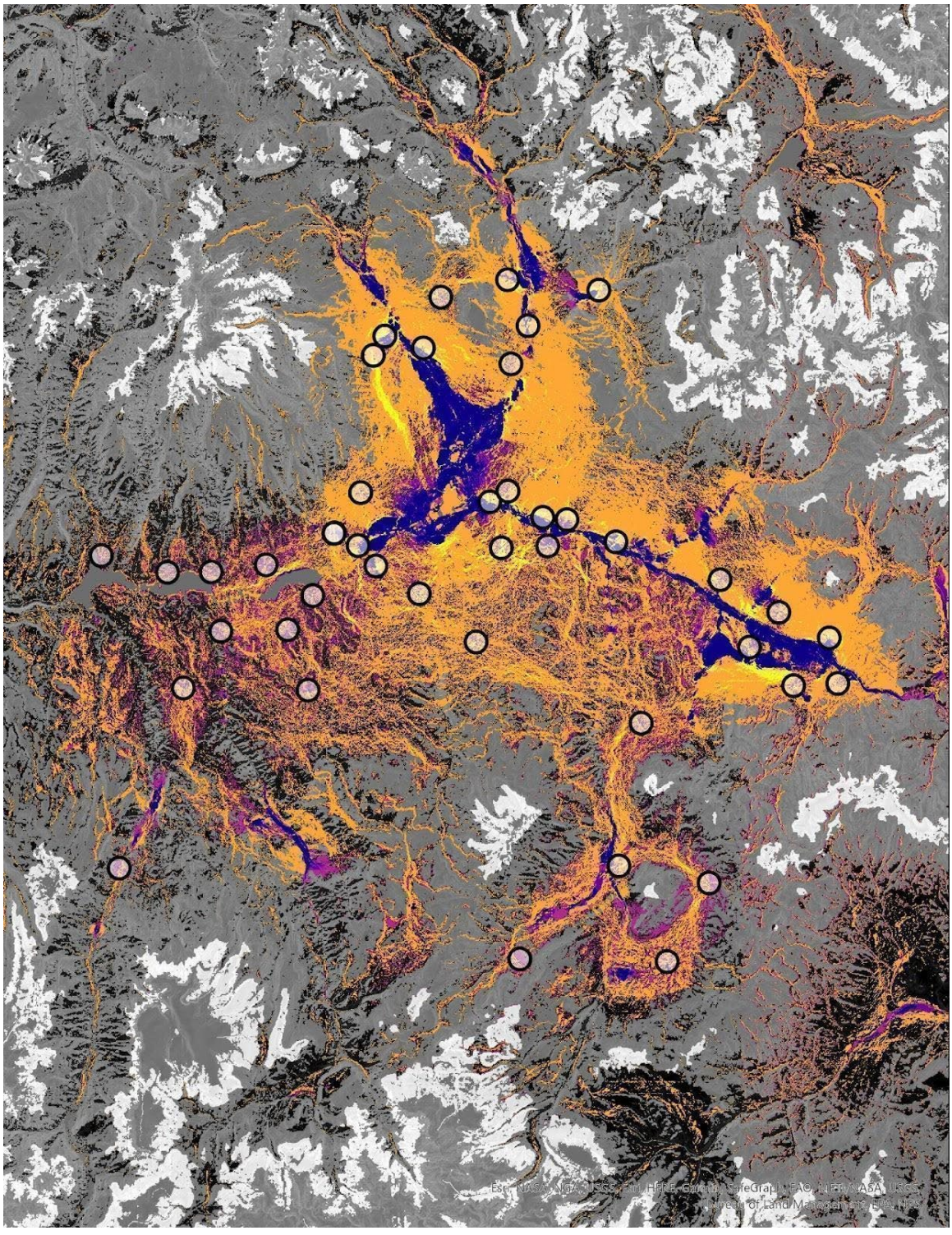


Figure 2.6b: The San Luis Valley: The map on the left shows a flow model using the finalized resistance surface and a subset of colonies distributed around the region. The map on the right uses the altered resistance surface that includes cultivated lands as a resistance surface.





Decreased Conductance — Increased Conductance

Figures 2.6 c and d: Each map shows changes in expected landscape conductance after cultivated lands are treated as high resistance landscape. Decreases in landscape conductance are shown as darker colors while lighter colors represent an increase of conductance. If there was little to no change in conductance, then the models are transparent showing the background resistance surface (low resistance to high resistance is shown as dark to light). **C)** The focus on the San Luis valley shows that much of the central portion of the valley is used for cultivation and now becomes a resistant core for the habitat area**. d)** The Gunnison Basin shows a similar result. Cultivated lands are found around the town of Gunnison in the center of the basin and cultivated lands border rivers and streams that run the east and north, providing a resistant barrier that may fragment the basin into regions.

2.5 DISCUSSION:

To conserve biodiversity, it is important to protect existing populations and maintain or restore habitat connectivity (Crooks and Sanjayan 2006; Resasco 2019). Connectivity is important for long-term population fitness by facilitating the exchange of individuals, allowing gene flow, and distributing adaptive variation across populations (Anantharaman et al. 2019; Luikart et al. 2019; Hohenlohe et al. 2021). For managers and conservationists, connectivity models can facilitate the understanding of how species and populations exist and move through the landscape (Sackett et al. 2012; St-Louis et al. 2014; Dutta et al. 2015). Connectivity models can be used to estimate the degree of population connectivity or risk of isolation, or to identify and protect habitat corridors important for connecting populations (Crooks and Sanjayan 2006; Walters and Schwartz 2020). Additionally, connectivity models can also be utilized to predict outcomes if a habitat corridor or population is created, re-established, or lost (Yumnam et al. 2014).

Isolation-By-Resistance:

The landscape resistance model was developed using methods from landscape genetics and circuit theory and validated with measures of genetic differentiation of two different genetic markers from colonies of Gunnison's prairie dogs. I found support that water, forest, and developed lands resist (limit) prairie dog movement. Additionally, the model predicted increased

prairie dog movement across flat or low sloping areas while steep slopes limited movement. The resistant features were incorporated into a comprehensive landscape resistance model that models how landscape influences prairie dog movement. I found that expectations of IBD and IBR are supported for *C.g. gunnisoni*, though IBR more closely describes genetic differentiation between colonies. The resistance surface developed in this study is used to model how Gunnison's prairie dogs are expected to move through the landscape, and to identify regions of high intrapopulation connectivity, barriers to interpopulation connectivity, habitat corridors, and possible isolation of populations.

Regions of Connectivity:

The range-wide conductance model shows many large expanses with moderate to high levels of continuously conductive landscape that are separated from each other by highly resistant landscapes (Figure 2.4). There appear to be at least three large and distinct population areas, each of which can be well defined by watershed boundaries. The Gunnison, Rio Grande, and the combination of the South Platte/Arkansas watersheds constitute three proposed population areas which are shown to have high intrapopulation connectivity and low interpopulation connectivity (Figure 2.4). The flat and rolling grasslands and shrublands of the San Luis Valley and Gunnison Basin contain large areas of suitable prairie dog habitat that is expected to facilitate dispersal. The Arkansas/South Platte basins have multiple smaller habitat regions including the upper Arkansas River Valley, South Park, and Wet Mountain Valley (Figure 2.1), each separated by low/moderate conductive landscape that is likely to reduce dispersal between the population areas but may not necessarily act as a barrier. The border between the Arkansas and South Platte watersheds does not appear to act as a significant barrier to prairie dog movement as many connectivity paths occur between these two watersheds (Figure 2.5b). A fourth population area is expected to occur in the

high elevation portion of the San Miguel watershed (colony 16/27). This area appears to be highly isolated from other population areas, is much smaller, and consists of habitat that is overall more resistant compared to other populated areas (Figure 2.7a).

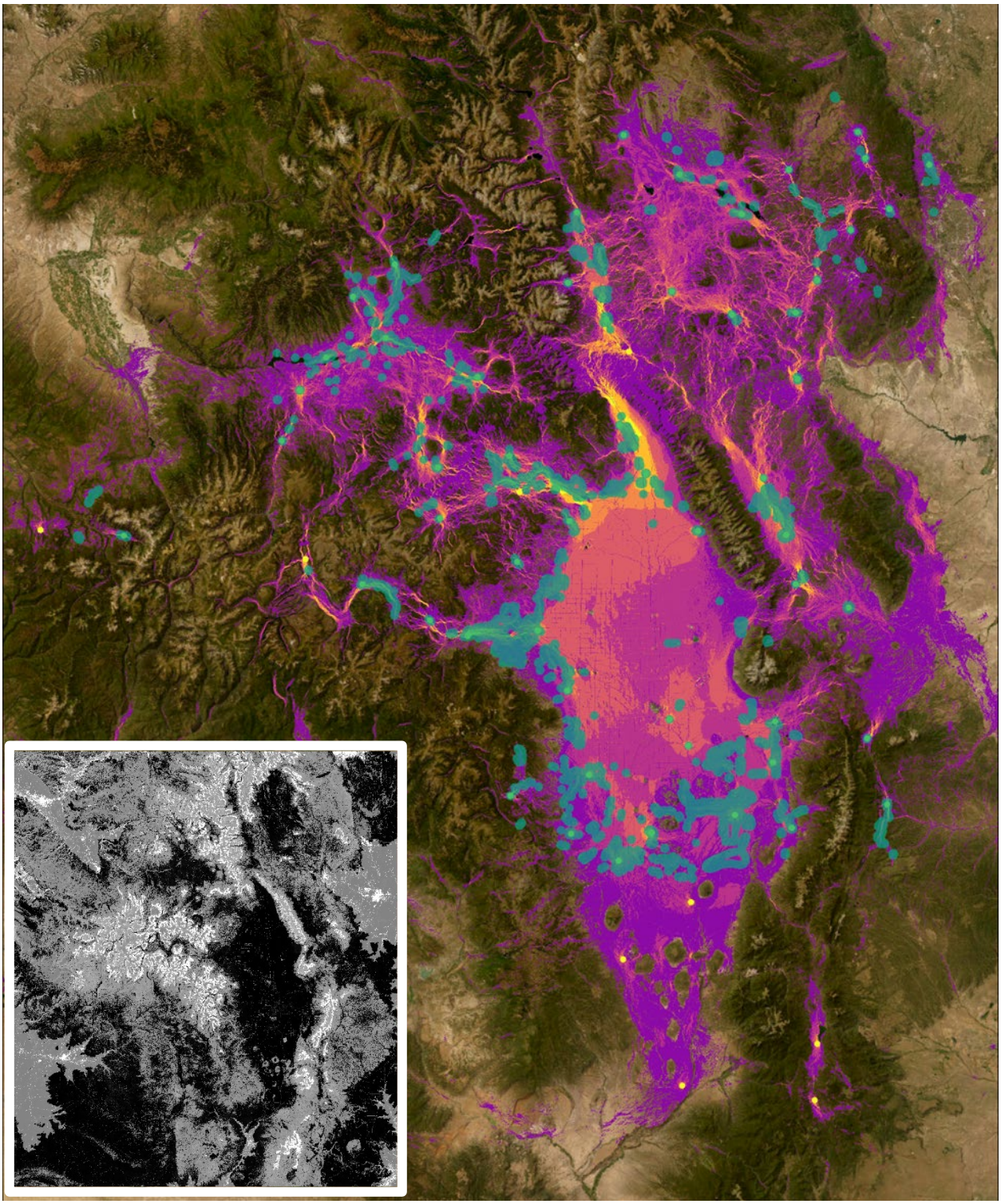


Figure 2.7: a) The flow model with potential Gunnison's prairie dog colonies (cyan dots). Colonies are only included for the state of Colorado and are located within low resistance regions of the habitat area, the darker shades shown in the insert (bottom left).

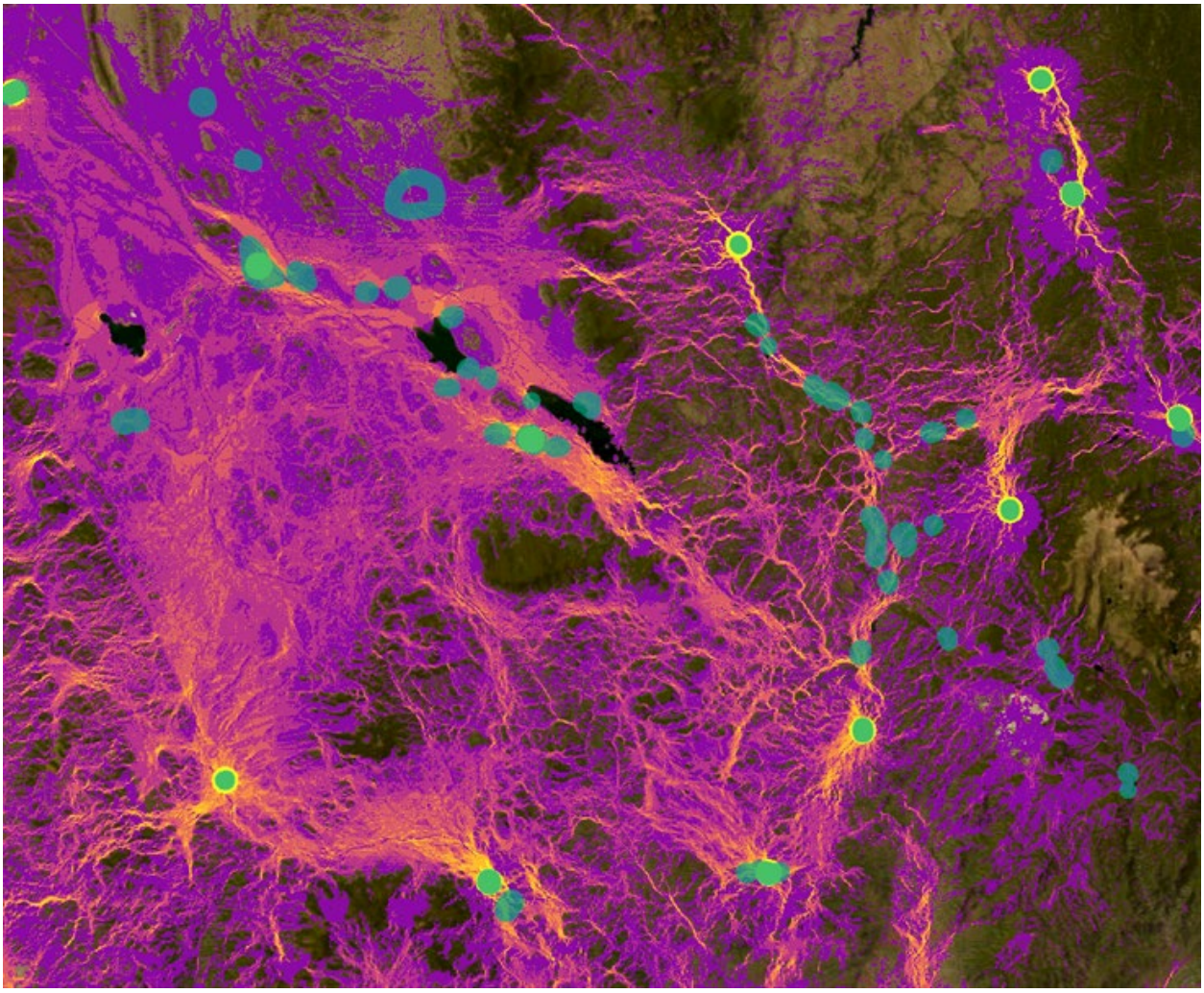


Figure 2.7: b) A focus on the region of South Park and the Pikes peak region shows how high topographical lands can concentrate paths of connectivity into thin corridors.

Low resistant habitat occurs in the large mountain valleys such as the San Luis Valley, Gunnison Basin, and South Park. There is strong overlap between colony location and high conductive landscape (Figure 2.7). Colonies are often found near the edges of population areas, but often in areas where landscape connectivity is much higher than the surrounding habitat (Figure 2.4 and 2.6). These connectivity paths likely facilitate the colonization of habitat near population edges. Two prominent examples among our sampled colonies occur with colony #14 in Gunnison which is along a habitat corridor created by the Lake Fork River, and #15 in the Rio Grande, whose connectivity path closely follows a corridor along the north fork of the Rio Grande

River. Drainages may be important for providing low sloping habitat corridors that facilitate dispersal for prairie dogs, allowing them to colonize habitat patches in otherwise marginal habitat space. Drainage corridors have been observed to explain the movement of black-tailed prairie dogs on the shortgrass steppe (Roach et al. 2001). Drainage systems may be important corridors for Gunnison's prairie dog dispersal.

The region around South Park and Pikes Peak shows how landscape connectivity can predict where prairie dog colonies occur (Figure 2.7b). The area between South Park, CO and Pikes Peak is a mixture of low forested and meadow habitat in topographic landscape. The flow model predicts a web of thin connectivity corridors that span the region and nearly every prairie dog colony in the area is located along a portion of the landscape that is expected to facilitate connectivity. Refined habitat models such as these could be useful in developing a habitat model for *C.g. gunnisoni* and for predicting locations of occupied or extirpated colonies. This model does not include information about soil types, so soils that provide poor prairie dog habitat, such as those that are sandy (The Great Sand Dunes) or rocky should be excluded for predicting possible prairie dog habitat (Seglund et al. 2005).

Interpopulation connectivity:

Mountain ranges are shown to be significant barriers to *C.g. gunnisoni* dispersal. However, there are specific areas where prairie dogs may be able to move between populations through lower resistance mountain passes. Along a portion of the Rio Grande/Gunnison watershed boundary are the Cochetopa hills, a region that has relatively low elevation mountains (Figure 2.5a; large, circled area). Colony 5, in the Rio Grande and the nearby colony 6, in the Gunnison area, show low levels of genetic differentiation ($F_{ST} = 0.10$), compared to other colony pairs in separate population areas

(Table 2.3), indicating evidence of recent gene flow occurring between the populations. The Cochetopa Hills provide several potential habitat corridors that prairie dogs may have used to disperse between the two regions. Another potential region of interpopulation gene flow between the Gunnison and Rio Grande is near Spring Creek Pass (Figure 2.5a; small, circled region), though the length and narrowness make this a less viable corridor. Poncha Pass (Figure 2.5b; circled area) connects the northern Rio Grande and southwest Arkansas areas, another likely corridor prairie dogs may have been able to disperse across. Paths like these may provide explanations how prairie dogs may have been able to move between population areas.

Habitat Conversion:

While urban lands, which make up 1.7% of the land cover were included in our model, cultivated lands (2.6%) were not. Prairie dogs are often removed from lands used for crops, hay, pasture, and ranching (Roemer & Forrest 1996; Figure 2.1). Flat, fertile lands which provide high quality prairie dog habitat are also desired for agricultural production. If prairie dogs are discouraged from establishing colonies on these lands, it could lead to increased fragmentation of populations in regions that are expected to provide excellent habitat and high connectivity in the core of many population areas.

Using the altered resistance model to include cultivated lands as a high resistant land type resulted in a shift in connectivity across in both the San Luis Valley and Gunnison Basin (Figure 2.6). The largest declines in connectivity occurred within the central regions of each population area where high agricultural activity occurs. Some of the landscape that was predicted to be highly conductive occurred over lands that have been converted for agriculture. If these lands exclude or act as a resistant surface to prairie dog movement, it could alter the degree of connectivity across

each population area. In the agricultural model, connectivity is predicted to be highest surrounding agricultural areas, altering predicted connectivity paths across each population area. Cultivated lands that were initially expected to be important corridors of connectivity, may reduce connectivity, and increase population fragmentation. The loss of core population areas as habitat may contribute to fragmentation, isolation, and disruption of region-wide demographic connectivity. More research would be needed to directly test the effect agriculture has on prairie dog connectivity. Genetic information or animal tracking methods could be used to evaluate the extent agriculture impacts population connectivity and a framework, such as the spatial absorbing Markov chain (SAMC) framework (See Fletcher et al. 2019) and be used for accounting of both movement and mortality risk if individuals that disperse across agricultural lands or other habitat types. For now, managers should consider potential implications in developing management strategies in regions with high agricultural activity.

2.6 **Conclusion:**

The use of resistance surfaces with programs like Circuitscape can be valuable for visualizing paths individuals of a species may use to move across habitat space. Additionally, connectivity models provide the ability to test or predict how connectivity will change after altering the landscape, whether it's making habitat unusable, constructing a barrier, or restoring habitat. These predictions can be valuable for evaluating management options and predicting consequences. This can be particularly informative for estimating historic, current, or future gene flow may occur as the landscape changes. These considerations and how they are addressed may have lasting consequences on populations and distributions of a species of conservation need.

CHAPTER 3

DESCRIBING GENOMIC VARIATION AMONG GUNNISON'S PRAIRIE DOGS AND IMPLICATIONS FOR CONSERVATION MANAGEMENT

3.1 ABSTRACT

Species of conservation concern often exist in fragmented habitats and experience low population numbers. Population connectivity is important to facilitate exchange of individual and genetic diversity is important for the long-term viability of populations. Fragmentation can allow genetic drift to erode the genetic diversity and the adaptive potential of a population. *Cynomys gunnisoni gunnisoni* is a subspecies of Gunnison's prairie dog that inhabits the montane regions of south-central Colorado and north-central New Mexico. Eradication campaigns, loss of habitat, and disease have resulted in large population declines for all prairie dog species. Today, *C.g. gunnisoni* exists in a patchwork of colonies among large mountain valleys and basins separated by impassable mountain ranges. To facilitate conservation and management efforts of the subspecies, I used genomic information to characterize patterns of genetic structure. High genetic differentiation among colonies shows there are multiple clusters of colonies that are spatially and genetically separated. Genetic differentiation increases with increasing distance between colonies. These results, in the context of the historical pressures on prairie dogs, suggest populations have been subject to isolation and fragmentation. Management actions may have to focus on elevating the genetic connectivity of prairie dog populations to prohibit reduction of population fitness by genetic deterioration and other processes, perhaps using assisted migration strategies.

3.2 INTRODUCTION:

The identification and management of population units is important for the successful conservation of biological diversity (Paetkau 1999). The conceptualization and delimitation of biological groups, from the species to intraspecies designations, remains a contentious, but essential, strategy for the preservation of genetically and demographically distinct populations of individuals (Waples 1991; Supple and Shapiro 2018). Population units delimited for conservation purposes are known as conservation units (CU) (Fraser and Bernatchez 2001). A CU is a general category for any group of organisms or populations that is delineated for one (or more than one) conservation purpose (Fraser and Bernatchez, 2001; Funk et al. 2012, 2019). The management unit, MU, is a commonly designated CU that is developed for management purposes (Funk et al. 2012). The decisions for how to establish a MU can vary with the management priority, but MUs are generally considered independently demographic subpopulations with internal growth rates dependent on local birth and death rates (Moritz 1994; Palsbøll et al. 2007). Multiple MUs can be established for the management of species. The designation of MUs facilitates the efforts of local wildlife managers for specific goals such as tracking population distribution, demographic trends, or managing harvest numbers for game units and fisheries (Palsbøll et al. 2007; Schwartz et al. 2007).

For imperiled species, the designation of population units is an important first step in evaluating conservation concerns for species and populations for the purpose of guiding management efforts (Fraser and Bernatchez, 2001; Funk et al. 2012, 2019). Advances in next generation sequencing has allowed for thousands of polymorphic loci to be used for providing

detailed information for biologists to evaluate relationships among geographically separate groups of non-model species (Allendorf et al. 2010; Supple and Shapiro 2018; Hohenlohe et al. 2021). Genetic information can provide insight into how populations are structured, where gene flow occurs, and measure genetic variation within and between groups (Funk et al. 2019; Hohenlohe et al. 2021).

For imperiled species, a conservation goal is to maintain the maximum amount of genetic variation a species has to adapt to environmental pressures (Funk et al. 2012). Measures of diversity, such as nucleotide diversity, heterozygosity, allelic diversity, and effective population size are important indicators of the capability for a population to adapt to future environmental change (Frankham 1996; Lonsinger et al. 2018). Observed heterozygosity (H_O) is the average proportion of heterozygous sites among individuals in a population. Expected heterozygosity (H_E), or a gene diversity index (Nei 1973), is the average proportion of heterozygotes per locus in a randomly mating population or the expected proportion of heterozygous loci in a randomly chosen individual.

Allelic diversity is a measure of the average number of alleles per locus and is more sensitive to losses of population size than is heterozygosity as it is concerned only with the presence of alleles at each locus and not the frequency of alleles as is heterozygosity (Allendorf and Luikart 2007; Allendorf et al. 2022). Allelic richness is often used in place of allelic diversity as sample size can heavily influence allelic diversity results. Allelic richness uses a rarefaction method to account for sample size when estimating allelic richness at each locus and is informative to estimate the amount of allelic variation within a population (El Mousadik and Petit 1996). Maximizing the conservation of as much genetic variation as possible and facilitating the spread of genetic variation among populations is important to increase the genetic variation and viability

of long-term persistence for groups of individuals (Hilborn et al. 2003; Funk et al. 2019; Allendorf et al. 2022).

C. gunnisoni is composed of two subspecies, a plains-dwelling form, *C.g. zuniensis*, and a montane form, *C.g. gunnisoni* and each could warrant consideration of listing under the ESA (Hollister 1916; Pizzimenti & Hoffmann, 1973; USFW 2013; Sackett et al. 2014). The long-term persistence of *C.g. gunnisoni* is of higher concern due to the subspecies' low population numbers and factors like disease, habitat loss and fragmentation, and eradication continue to threaten populations (Seglund and Schnurr 2010; USFW 2013). In this study, I used genetic markers to calculate genetic diversity in colonies and explore the genetic structure of *C.g. gunnisoni* colonies.

3.3 Methods:

Sample collection, DNA extraction, and sequencing were conducted as in Chapter 2, though, only colonies with whole genome data are used in this analysis (Figure 3.1). There are a few key changes from the population numbering from Chapter 2 to Chapter 3. In Chapter 2, colony 15 has been assigned as Colony 4/RH in Chapter 3. Colonies 7 and 8 in Chapter 2 are combined into colony 8/B18 in Chapter 3 (See Table 3.1 for comparisons between population IDs of Chapter 2 and 3). This was done for organizational purposes in the case of colony RH, and to combine populations with low sample size and high genetic similarity in the case of the colonies being identified as 8/B18. Additional information about each sampled colony is provided in Table 3.2

Table 3.1: Showing how the colony identification changes between Chapter 2 and 3. Colonies that are labeled differently between Chapter 2 and 3 are shaded. Additionally, the individuals from colonies 7 and 8 in Chapter 2 were combined into a single site named 8/B18.

Chapter 3 Population ID 1	Chapter 3 Population ID 2	Chapter 2 Population ID
DR	1	1
CM	2	2
EM	3	3
RH	4	15
Ant	5	4
SPGL	6	5
B09	7	6
B18	8	7
		8
HK	9	9
MR	10	10
KM	11	11
Pow	12	12
CR	13	13
Gate	14	14
TE	15	16

Table 3.2 Information about each sampled colony. The watershed, elevation, and a description of the expected level of isolation, habitat composition, and the size of the colony when it was sampled are provided.

ID1	ID2	Elevation (m)	Watershed	Notes
DR	1	2672	Arkansas	Expected Isolation- moderate/high; Colony is in an area surrounded by forest and dispersal paths are unknown. Many known colonies exist within 5-10km. Habitat: Montane meadow habitat surrounded by pine forest. Size at time of sampling: Large and populated colony, died off the year after sampling occurred. 20+ acres
CM	2	2641	S. Platte	Expected Isolation- Low. a few colonies occur in this region of South Park Habitat: high elevation grassland Size at time of sampling: Large and populated colony 30+ acres
EM	3	2615	S. Platte	Expected Isolation- Low. a few colonies occur in this region of South Park Habitat: high elevation grassland Size at time of sampling: Large and populated colony 40+ acres
RH	4	3132	Rio Grande	Expected Isolation- High. Only one other small colony was in the area and dispersal is limited to much of the surrounding habitat. Habitat: montane meadow. Highest elevation colony I located across the entire range. Size at time of sampling: Large and populated colony. 30+ acres
Ant	5	2427	Rio Grande	Expected Isolation- Low. many colonies exist in the surrounding region. Habitat: mountain grassland. Size at time of sampling: Large but density was variable. Plague was likely going through the colony at the time of trapping- 1 individual that had plague was found (CPW communication)
SPGL	6	2674	Rio Grande	Expected Isolation- moderate. Colonies occur in low density across the area which contains montane meadow interspersed with forests. Habitat: montane meadow Size at time of sampling: moderately large and populated colony. 20+ acres
B9	7	2817	Gunnison	Expected Isolation- moderate: a few other colonies are known in the region, but they are at low density considering the size of Cochetopa park and the amount of available habitat. Habitat: montane grassland, rolling hills. Size at time of sampling: Large but low-density colony.

Table 3.2 continued:

B18	8	2392	Gunnison	Expected Isolation- Low, multiple colonies are found within 5km. Habitat: grassy patch in sagebrush habitat. Size at time of sampling: Large and populated colony. 30+ acres This population contains individuals from two colonies 2km apart.
HK	9	2895	Gunnison	Expected Isolation- moderate/high. Habitat: montane meadow on a ridge surrounded by sagebrush habitat. Size at time of sampling: somewhat small colony small/moderate. 15+ acres
MR	10	2502	Gunnison	Expected Isolation- moderate a few colonies are found along a drainage corridor. Habitat: montane meadow surrounded by pasture and sagebrush habitat. Size at time of sampling: somewhat small colony, recovering from a population collapse. small/moderate. 10+ acres
KM	11	2647	Gunnison	Expected Isolation- moderate a few colonies are found along a drainage corridor. Habitat: montane meadow surrounded by pasture and sagebrush habitat. Size at time of sampling: small/moderate. 20+ acres
Pow	12	2511	Gunnison	Expected Isolation- moderate, marginal habitat but other colonies may exist in the region. Habitat: low density sagebrush habitat within a small valley. Size at time of sampling: large but low-density colony. 60+ acres
CR	13	2556	Gunnison	Expected Isolation- moderate a few colonies are known in the region, but low considering abundant habitat space. Habitat: grassy patch within sagebrush habitat. Size at time of sampling: somewhat small but dense colony. 15+ acres
Gate	14	2503	Gunnison	Expected Isolation- low, this is the furthest known colony along a sparsely populated drainage corridor, bordered by a highway, river and forested slope. Habitat: former pastureland surrounded by sagebrush and forests. Size at time of sampling: moderately sized colony. 20+ acres
TE	15	2663	San Miguel	Expected Isolation- high. Other individuals have been spotted near the colony, but no other distinguishable *C.g. gunnisoni* colonies are known in the San Miguel region. *C.g. zuniensis* populations exist within 50 km to the west. Habitat: mixture of sagebrush, pasture, riparian. Telluride valley floor is along the San Miguel River. Size at time of sampling: large colony. 40+ acres but collapsed to 1-2 dozen individuals in 2019.

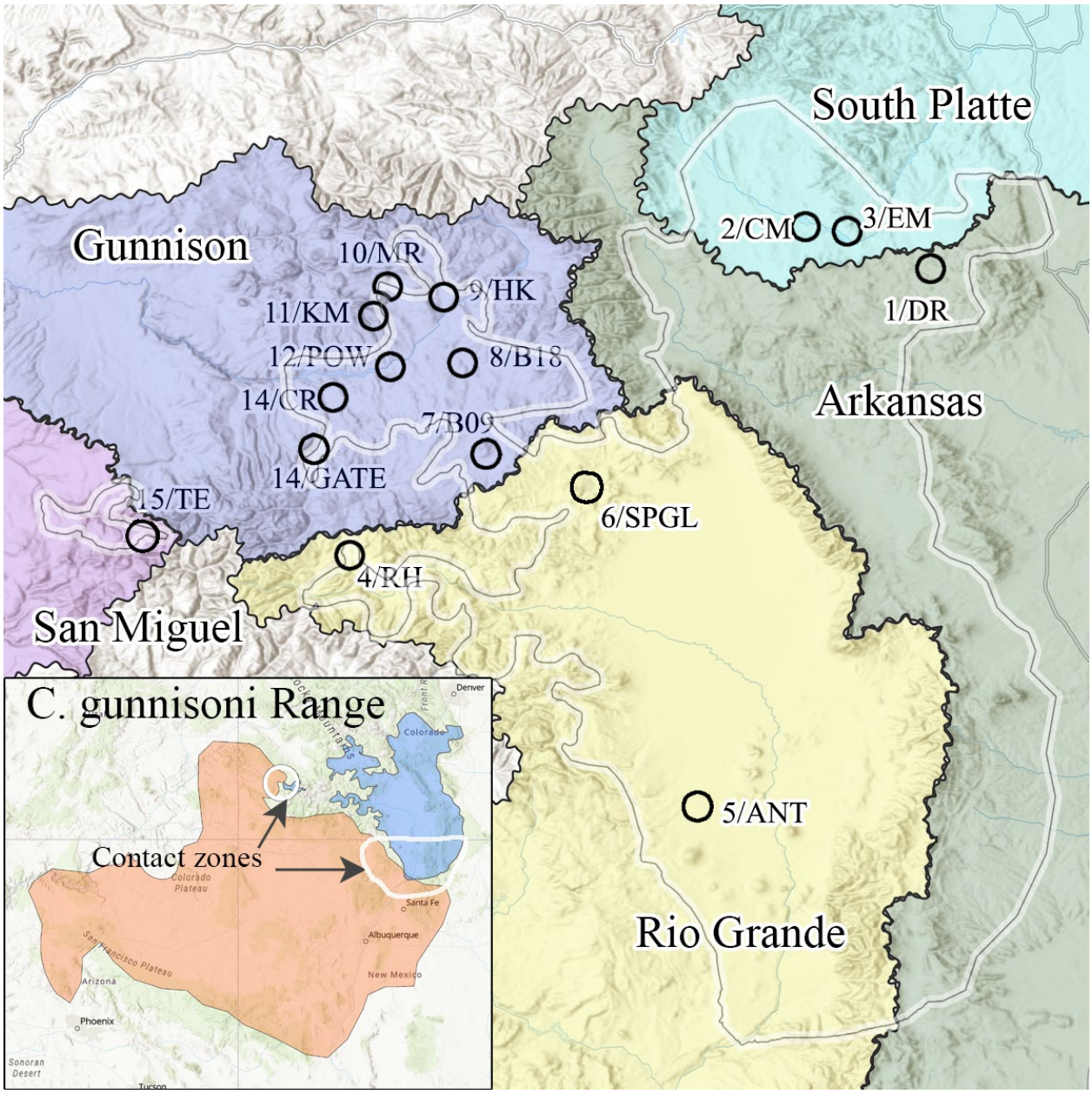


Figure 3.1. A map showing the range of *C.g. gunnisoni*. The insert (bottom left) shows the approximate range of *C. gunnisoni* including C.g. *gunnisoni* -blue- and *C.g. zuniensis* -orange. There are two separate contact zones between the subspecies. The range of *C.g. gunnisoni* is outlined (white) in the background and sampling locations are shown as open circles. *C.g. gunnisoni's* range spans 5 major watersheds labeled: Rio Grande- yellow, San Miguel- purple, Gunnison-blue, Arkansas- green, South Platte- Cyan. Sampling locations are represented by open circles.

Genotype filtering processes were altered to remove additional uncertain genotypes for this study. As in Chapter 2, a self-similarity filter, according to Lynch *et al.* 2016, was run on the Gunnison's prairie dog reference genome (Tsuchiya et al. 2020) to soft-mask genomic regions containing stretches of greater than 500 bp in length with greater than 97% identity to other regions. This removes extensive repetitive regions of the reference genome and improves genotyping accuracy. Genotypes were called by aligning reads to the filtered reference genome using bwa-mem (Li 2013), followed by *samtools* mpileup (Li et al. 2009) and *bcftools* call (Li 2011), and variant sites were retained. An alignment-depth histogram was constructed using all remaining individuals aligned to the reference genome. Sites in the genome with an alignment depth of between 69 and 171 were inferred to be safely in the single-copy portion of the genome, and all other sites were discarded from analysis. Samples that were sequenced under an average sequencing depth less than 1X that of the genome size were removed from the analysis to improve coverage per site percentage and increase certainty of inferred phylogenetic relationships. The VCF was then filtered for completeness – variants containing fewer than 50% of individuals genotyped at that location were discarded. Variants with low quality score (<999) and multiallelic variants were discarded (using VCFtools v.0.1.16; Danecek et al. 2011).

At this filtering step, I used two different filtering options to create three different data sets for specific analyses. First, because low frequency alleles may represent PCR errors, sites with a minor allele frequency (MAF) of less than 5% were discarded. This data set, called -**VCF1**- is used for all remaining analyses. With 160 individuals and sites approaching 50% coverage, a rare allele would need to occur within 16 individuals. With a maximum number of individuals per colony of 14 and alleles occurring in as few as 50% of individuals, these filtering methods would remove most rare alleles, including those that are unique to individual colonies. To analyze private alleles (alleles found within a single colony and nowhere else), I created a second variant sites dataset, called **VCF2** that used different genotype filtering methods. I used the same genotype data and filtering methods up until the MAF filtering step. At this point, I did not filter by MAF, but instead filtered out alleles with a minor allele count of less than three. This may result in an increased number of false variants, but VCF2 will be primarily used for measuring the number of unique alleles in each colony.

Genetic Structure:

I used multiple tests to examine the population structure of *C.g. gunnisoni.* There are a number of possible population groups ranging from the smallest unit at the colony level to the largest being the entirety of the subspecies. I performed analyses using Bayesian clustering methods, multivariate analyses, and developed a neighbor net tree (Figure 3.2). I used the Bayesian clustering algorithm, FastSTRUCTURE (Raj et al. 2014), to estimate the number of ancestral populations in the *C.g. gunnisoni* range. FastSTRUCTURE, similar to the program STRUCTURE (Pritchard et al. 2000) it is modeled on, uses Markov chain Monte Carlo (MCMC) simulations to estimate the proportion of an individual's ancestry is part of one or more genetic clusters (K) and works to minimize the amount of linkage disequilibrium within clusters (Pritchard et al. 2000; Raj et al. 2014). Because MCMC methods may vary from run to run, multiple runs of K are performed (Gilbert et al. 2012). High genetic structure will separate individuals and populations into distinct genetic clusters (Figure 3.2). To estimate the best number of genetic clusters I ran 10 iterations for each value of K from K=1 to K=18. I inferred the best-supported value of *K* using the Choose K method and by estimating the smallest number of model components that accounts for the ancestry in the sample (Raj et al. 2014) as implemented in structureSelector (Li and Liu 2018). CLUMPAK was used to assign individuals to genetic clusters using q-values and to create and visualize STRUCTURE plots (Kopelman et al. 2015).

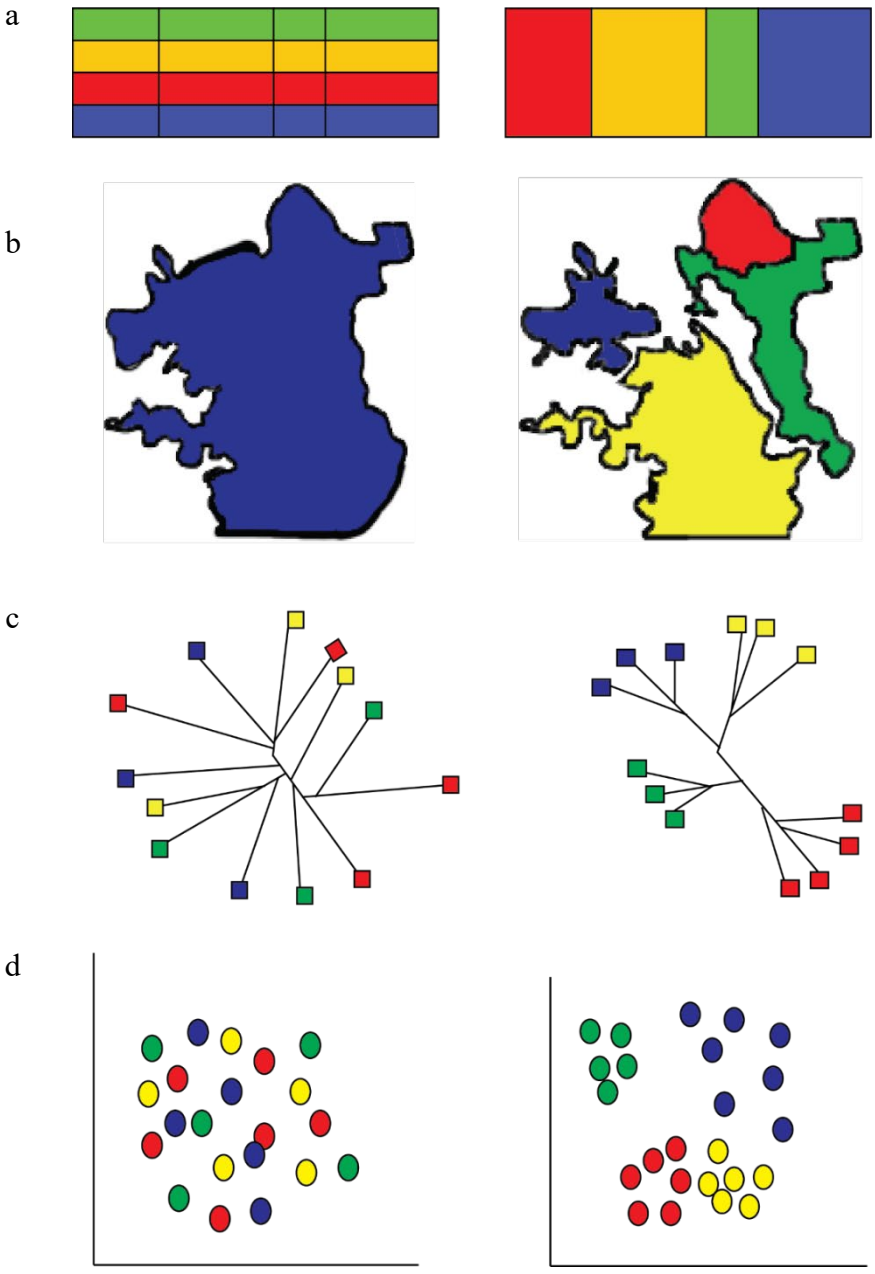


Figure 3.2: Predictive models exemplifying differences in results showing low vs. high population structure. Examples include results from a) STRUCTURE plot, b) representation of possible populations on the landscape, c) unrooted phylogenetic network, and d) similarities from axis one and axis two of a principal component analysis (PCA).

Next, I examined patterns of genetic divergence and similarity among prairie dog colonies. I implemented a multivariate approach using principal components analysis (PCA) and a discriminant analysis of principal components (DAPC) with the Adegenet R package (V2.1.5; Jombart et al. 2010). DAPC uses principal component information and minimizes within group variation while maximizing between group variation. To further explore fine scale patterns of genetic similarity I performed additional DAPC analysis on subsets of data containing colonies that clustered together in PCA and DAPC axes 1 through 3.

I examined phylogenetic histories among prairie dog colonies using a phylogenetic network in the program Splitstree4 with the uncorrectedP method that computes distances using the proportion of positions that are different between two sequences (Bryant & Moulton 2004; Huson & Bryant 2006). I also evaluated the relationships between *C.g. gunnisoni* colonies and *C.g. zuniensis* in an exploratory analysis where I incorporated genomic data from *C.g. gunnisoni* with that of six individuals located in the New Mexico region of *C.g. zuniensis* range. This sequence data was used to develop a VCF using the genotype calling and filtering methods from Chapter 2 (*C.g. zuniensis* genomic sequences provided by Dr. Loren Sackett). The resulting genotypes data were used to create a phylogenetic network in Splitstree4 to visualize the relationships between *C.g. gunnisoni* colonies and the *C.g. zuniensis* subspecies. This was included in this study to provide information about relationships between the *C. gunnisoni* colonies and the *C.g. zuniensis* subspecies.

Genetic diversity and differentiation

To characterize population genetic differentiation among colonies of *C.g. gunnisoni,* I calculated two different measures of genetic differentiation between pairs of colonies: pairwise F_{ST} (HEIRFSTAT R package; V0.5-7) and Jost's D (mmod R package V. 1.3.3; Jost 2008; Winter

2012; Jost et al. 2018). I calculated the correlation coefficient between Jost's D and F_{ST} to evaluate if genetic differences between populations are similar for each differentiation statistic (Verity & Nichols 2014). I calculated the proportion of alleles shared between each colony using the dartR package (Gruber et al. 2018).

Genetic diversity and differentiation

Tests of isolation-by-distance (IBD) and isolation-by resistance (IBR) were performed to evaluate how each explains genetic differentiation. I calculated pairwise values of geographic distance in ArcGIS Pro and pairwise resistance values were calculated from Circuitscape using the resistance surface from Chapter 2. Mantel tests were used to evaluate how geographic distance and pairwise resistance explain genetic distance (Slatkin's linearized F_{ST}) using the vegan R package (version 2.6-2; Oksanen 2013).

To compare genetic diversity estimates among populations, I calculated multiple measures of genetic diversity for each colony. To calculate the proportion of heterozygous sites within each colony, I calculated the observed heterozygosity (H_o) and the expected heterozygosity (H_e) with the R package HIERFSTAT (Goudet 2005). Expected heterozygosity is a measure that calculates the average proportion of heterozygous sites per locus based on allele frequencies assuming Hardy-Weinberg equilibrium in a population that is randomly mating (Allendorf et al. 2022; Nei 1987). I calculated allelic richness (A_R) with the R package HIERFSTAT (Goudet 2005), which estimates the rarefied allelic counts per locus and population and adjusts for variation in sample sizes. Allelic richness is more sensitive to losses of genetic variation and caused by reductions in population numbers than measures of heterozygosity (Allendorf 1986; 2022). Finally, I calculated the proportion of shared alleles within colonies using the dartR R package (Gruber et al. 2018).

To obtain an estimate of how much unique allelic variation occurs within colonies, I measured the number of private alleles within each population using both VCF files -VCF1 and VCF2- with the R package HIERFSTAT (Goudet 2005). Private alleles are alleles that are only found within a single population. Populations with higher numbers of private alleles are expected to have lower rates of gene flow to neighboring populations (Barton and Slatkin 1986), suggesting that isolated populations may retain large amounts of unique genetic diversity. To confirm, I evaluated whether the distance from the nearest neighboring colony may influence the number of private alleles within a colony.

Colony distribution on the landscape:

To evaluate how colonies cluster on the landscape I chose to focus my efforts on the Gunnison Basin. The Gunnison basin is the most well represented region in this study and colonies in the area have been well documented. I obtained colony locations from the CPW Species Activity Mapping (SAM) data. I removed colonies I determined were not active based on personal observations between 2015 and 2022. In ArcGIS Pro, I buffered the boundary of each colony location to three kilometers (average prairie dog dispersal distance: Garrett and Franklin 1988; Antolin et al. 2006) and merged overlapping polygons. I overlaid the resulting shapefile onto the flow model developed in Chapter 2 (Figure 2.6a: using the resistance surface the incorporated cultivated lands as high resistance). Non-overlapping polygons are less than six kilometers apart.

3.4 Results:

In 2017, I trapped 300 prairie dogs from 12 colonies distributed across the range of *C.g. gunnisoni.* I chose colonies that provided a distribution of samples ranging from some that are in close proximity (i.e., MR-KM; EM-CM; B18-B05) to others that were expected to be isolated (i.e.,

TE; RH; Gate). I sampled three colonies in the Rio Grande and San Luis Valley region, nine colonies in the Gunnison Basin, and one colony outside the town of Telluride Colorado. In addition, I obtained 238 blood samples by the CPW. These included samples from two colonies in South Park and two colonies in the Gunnison Basin during research conducted in 2017. Additionally, I obtained archived blood samples from two colonies near the town of Florissant, CO, collected by the CPW during a 2014 study. Genotype filtering resulted in a VCF file that contained variant genotypes. VCF1, resulted in 63,169 SNPs from 192 individual prairie dogs. VCF2, which filtered sites using a minor allele count of 3 instead of a minor allele frequency of 0.05, resulted in 118,013 SNPs from the same individuals and colonies as in VCF1.

Genetically divergent populations:

Analyses from FastSTRUCTURE, PCA, DAPC, and neighbor joining trees provided multiple ways of evaluating genetic divergence between groups of individuals. The Bayesian clustering algorithm implemented in FastSTRUCTURE (Figure 3.3) and analyzed in CLUMPAK, provided multiple predictions for how individuals were assigned to each ancestral population of K (Figure 3.3). Between K=1 and K=7, there was a continuous breakdown of colonies into different genetic groups. First, there was a strong separation between sites 1-3 (green; from the South Park region) and all other sites. Next, colony 15/TE (purple; San Miguel) clustered as another distinct population. At K=4, colony 4/RH (red; Rio Grande) was shown to be another unique genetic cluster, though colonies 5-7 were a mixture of three different ancestral populations. At K=5, two Rio Grande colonies (yellow) clustered together and separated from colony 7/B9, which was associated with the other Gunnison colonies. At K=7, and beyond, further breakdown of the genetic clusters occurred among the Gunnison Basin colonies. The next prominent genetic group, -colored cyan in Figure 3.3- appears to associate with colonies in the southern portion of the

Gunnison basin and blue represents colonies in the north. Colonies 7/B18 and 12/Pow, which show a split in ancestry (Figure 3.3: K=7; shown as blue and cyan), are located near Tomichi Creek and the Gunnison River which are two drainage that run east to west and located between the northern and southern portions of the Gunnison Basin.

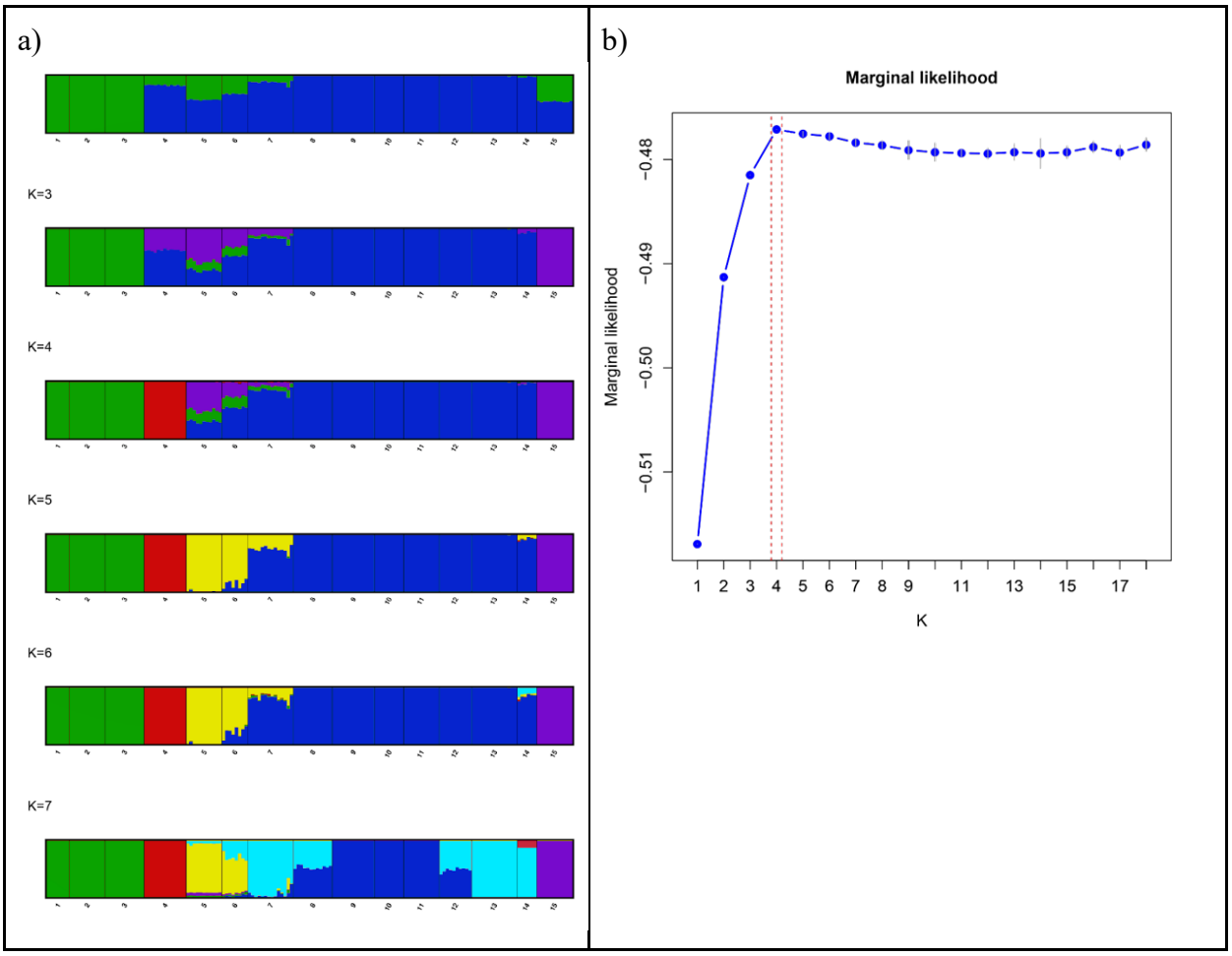


Figure 3.3: Results from FastSTRUCTURE. a) Structure plots from K=2-K=7. Colonies 1-3 (green) are from the South Platte and Arkansas watersheds. Colony 4 (red) is a single isolated colony in the Rio Grande Watershed. Colonies 5 and 6 (yellow at K=5+) are also in the Rio Grande watershed. Colonies 7-15 are in the Gunnison watershed (blue), though colony 7 is geographically near to colony 6. Colony 15 (purple) is in the San Miguel watershed, near the mountain town of Telluride CO. b) A maximum marginal likelihood is at K=4.

I calculated likelihood estimates for how well individuals partitioned out into the K number of genetic groups (Figure 3.3a) and revealed a steep increase in likelihood with increasing number of groups until K=4 (Figure 3.3b). Thus, the genetic data support recognition of 4 distinct

groups, agreeing with the choose K method for selecting the best "K" (Raj et al. 2014) that maximizes marginal likelihood at K=4.

PCA and DAPC analyses showed evidence for multiple genetically distinct groups, though some genetic clusters were more distinct than others (Figure 3.4). There was evidence for clear separation of the cluster of northeast colonies (FFB, EM, & CM) from other populations. Colonies 4/RH, 5/ANT, and 14/TE also showed distinct separation from all other populations along multiple axes. The Gunnison colonies clustered together, apart from colony 9/HK, which was slightly divergent in the DAPC plots.

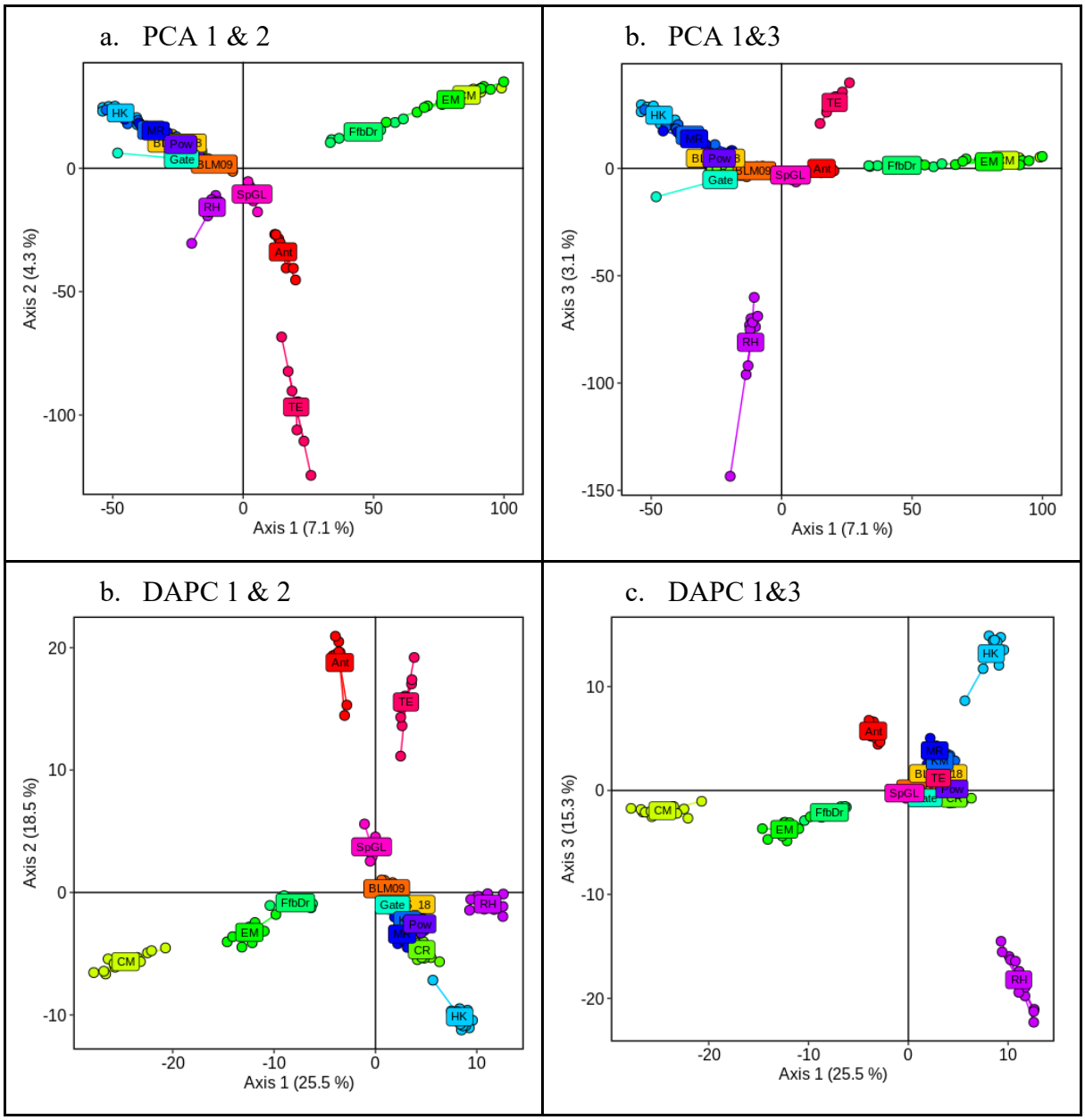


Figure 3.4: Visualization using genotype data from all colonies in an analysis of principal components, and a discriminant analysis of principal components are shown for axes 1, 2, and 3. Colonies located in the northeast portion of the range (green/yellow; DR, EM, CM) show strong divergence in each analysis. The colonies in the Gunnison region are clustered together, with only 9/HK showing some separation. Colony 15/TE and the three Rio Grande colonies, ANT, RH and SPGL, show varying amounts of clustering and separation depending on each axis.

I observed the variation in dissimilarity between colonies within each watershed group through visualization of DAPC plots that subset different regions (Figure 3.5). There were high amounts of dissimilarity occur within the Rio Grande colonies, moderate amounts in the Gunnison colonies, and low amounts in the colonies in the Arkansas/South Platte regions. The low amount of dissimilarity between colony 1/DR and either 2/CM or 3/EM is slightly surprising as the distance between these sites were 27 and 35 km, respectively, and the terrain between DR and South Park is forested and topographic compared to many colonies in the Gunnison Basin that show higher levels of dissimilarity but are geographically closer and occupy a more conductive habitat space.

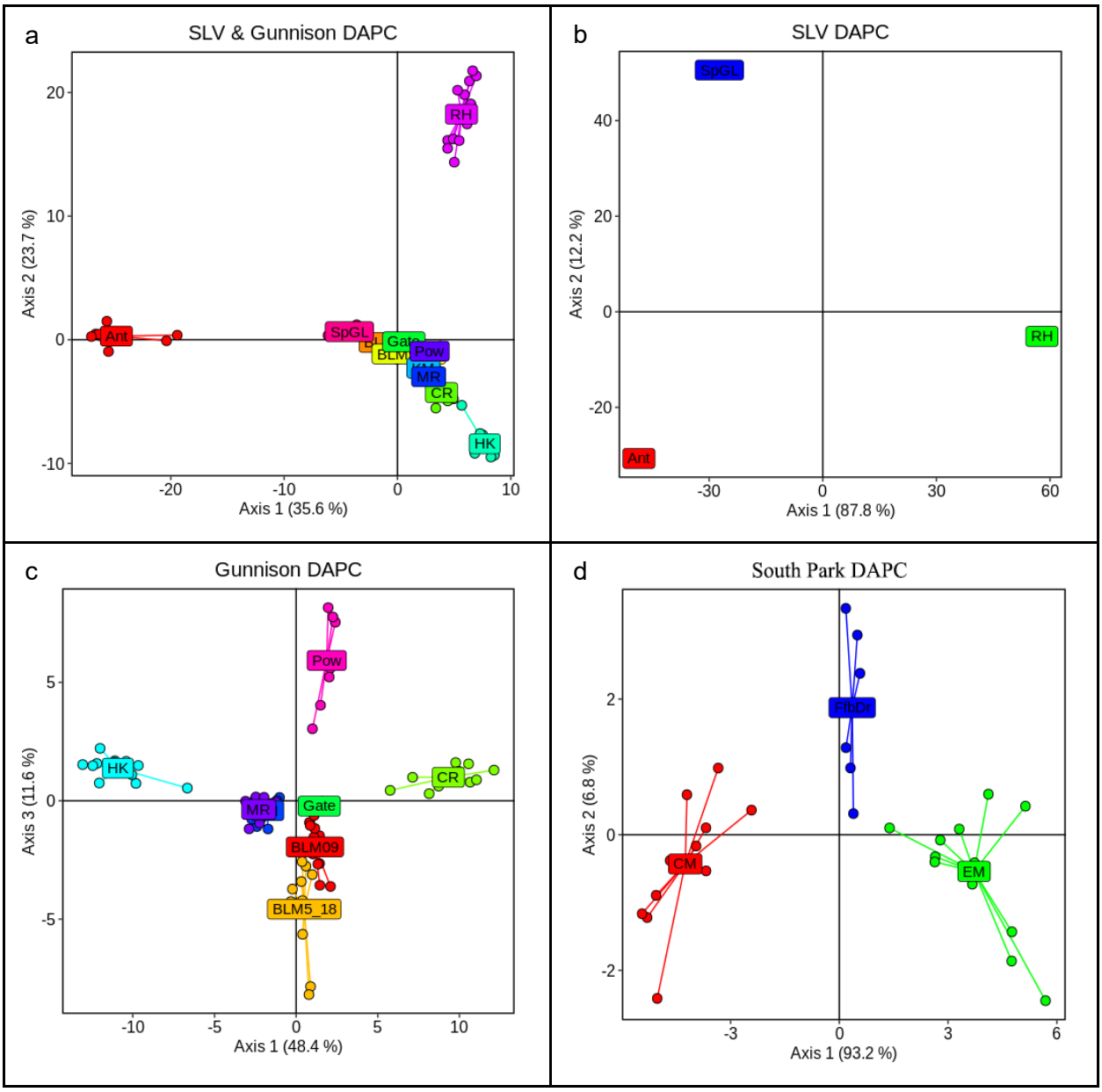


Figure 3.5: Visualization using genotype data from subsets of colonies in a discriminant analysis of principal components are shown for axes 1, 2, and 3. a) Gunnison colonies show high clustering while Rio Grande colonies are dissimilar. SPGL is clustered closest to the Gunnison colonies. b) there is high similarity between the Rio Grande colonies. c) Three colonies in the northern part of the Gunnison basin are shown toward the left of the plot. d) Compared to the other population areas, colonies in the Arkansas/South Platte watersheds are very genetically similar as there is little variation between colonies.

The phylogenetic network depicting the estimated phylogenetic relationships among individuals showed that most of the defined branches were made up of individuals belonging to the same colony (Figure 3.6). A few instances occur where individuals from multiple colonies are on the same branches, including the colonies in the north-eastern region (1-3) that are on a single branch that is distant from all other branches (green). Additionally, two pairs of colonies in the Gunnison basin (blue), 10/MR&11/KM and B18&B5 (both B18&B5 already combined into a single colony for this analysis) are also on the same branch. The three Rio Grande colonies (yellow/red) are distinct and separate branches. Within the Gunnison Basin (blue), branches are near to each other but separated except for the two pairs of colonies previously mentioned.

Pairwise measures of genetic differentiation, F_{ST}, range from 0.022 to 0.399 and values of Jost's D range from 0.010 to 0.168 (Table 3.3). The Pearson's correlation coefficient (r) between F_{ST} and Jost's D is 0.996, indicating a strong relationship between the two measures of genetic differentiation. The proportion of shared alleles between colonies ranges from 0.69 to 0.87 (Table 3.4).

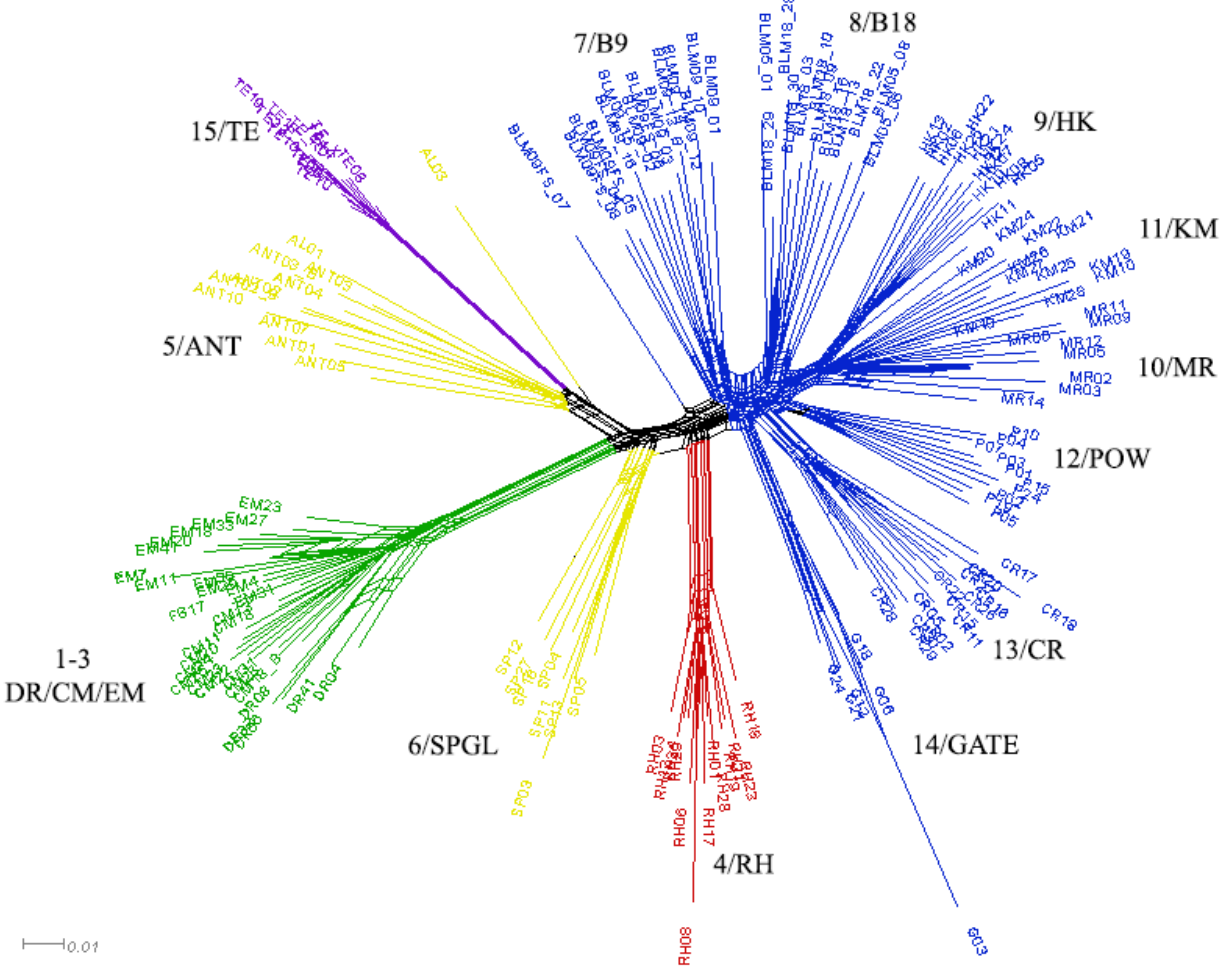


Figure 3.6: A phylogenetic network further shows relationships between individuals and colonies. Individuals are colored to represent proposed management groups. Blue shows colonies found within the Gunnison Basin. The left side of the tree are the northeast colonies (green) clustered, with little separation to distinguish colonies apart. The Rio Grande colonies (colored in yellow -6/SPGL & 5/ANT- and red -4/RH-) show slight associations to each other compared to other colonies, they do not show clustering as strong as the Arkansas/South Platter or Gunnison colonies.

Table 3.3: Pairwise Jost's D (top triangle) and F_{ST} (bottom triangle). Increased shading indicates higher relative values of each measure. Boxes represent watershed areas: From top left to bottom right: Arkansas & South Platte, Rio Grande, Gunnison, and 15/TE is the only colony from the San Miguel.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DR		0.019	0.014	0.148	0.101	0.108	0.119	0.134	0.154	0.148	0.143	0.136	0.139	0.168	0.166
EM	0.057		0.013	0.132	0.089	0.094	0.102	0.116	0.136	0.130	0.124	0.119	0.121	0.150	0.150
CM	0.065	0.048		0.132	0.089	0.096	0.102	0.116	0.136	0.131	0.124	0.119	0.121	0.151	0.151
RH	0.317	0.292	0.289		0.087	0.087	0.076	0.082	0.101	0.097	0.092	0.085	0.087	0.115	0.146
Ant	0.199	0.196	0.193	0.195		0.048	0.060	0.073	0.093	0.086	0.081	0.078	0.083	0.107	0.079
SPGL	0.229	0.223	0.217	0.211	0.105		0.052	0.064	0.083	0.074	0.070	0.068	0.072	0.101	0.112
B09	0.245	0.230	0.226	0.186	0.130	0.122		0.036	0.055	0.048	0.045	0.041	0.047	0.081	0.120
B18	0.267	0.252	0.249	0.197	0.153	0.144	0.085		0.040	0.034	0.030	0.034	0.044	0.080	0.134
HK	0.314	0.291	0.288	0.239	0.199	0.194	0.137	0.102		0.027	0.025	0.043	0.058	0.094	0.149
MR	0.283	0.274	0.271	0.223	0.173	0.160	0.110	0.078	0.073		0.010	0.036	0.052	0.087	0.147
KM	0.283	0.267	0.263	0.216	0.168	0.159	0.107	0.072	0.068	0.022		0.032	0.048	0.083	0.139
Pow	0.283	0.265	0.261	0.211	0.165	0.160	0.102	0.083	0.115	0.086	0.082		0.041	0.077	0.136
CR	0.290	0.267	0.264	0.212	0.179	0.172	0.117	0.110	0.148	0.127	0.119	0.110		0.078	0.139
Gate	0.352	0.329	0.322	0.276	0.218	0.237	0.192	0.187	0.227	0.198	0.196	0.198	0.197		0.165
TE	0.384	0.344	0.340	0.340	0.198	0.288	0.288	0.309	0.335	0.329	0.319	0.328	0.322	0.399	

Table 3.4: Top triangle: the proportion of shared alleles between populations while the diagonal shows the proportion of shared alleles between individuals within each population. Darker shades indicate lower amounts of shared alleles.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DR	**0.82**	0.85	0.86	0.71	0.74	0.74	0.73	0.71	0.70	0.70	0.71	0.71	0.72	0.69	0.71
CM		**0.80**	0.88	0.74	0.77	0.76	0.76	0.74	0.73	0.73	0.73	0.74	0.74	0.71	0.72
EM			**0.82**	0.74	0.77	0.76	0.75	0.74	0.73	0.73	0.73	0.74	0.74	0.71	0.72
RH				**0.82**	0.77	0.77	0.79	0.78	0.77	0.77	0.77	0.78	0.78	0.75	0.73
Ant					**0.76**	0.80	0.79	0.78	0.77	0.76	0.77	0.77	0.78	0.74	0.79
SPGL						**0.79**	0.80	0.78	0.77	0.77	0.78	0.78	0.78	0.75	0.75
B09							**0.78**	0.83	0.81	0.81	0.82	0.82	0.82	0.77	0.74
B18								**0.78**	0.84	0.83	0.84	0.83	0.83	0.77	0.73
HK									**0.80**	0.85	0.86	0.83	0.82	0.77	0.72
MR										**0.78**	0.87	0.83	0.81	0.77	0.71
KM											**0.79**	0.83	0.82	0.77	0.72
Pow												**0.80**	0.83	0.78	0.73
CR													**0.80**	0.78	0.73
Gate														**0.85**	0.71
TE															**0.88**

Genetic clusters appeared to follow a hierarchical structure. Individuals from a colony were highly clustered and colonies that occurred in geographically proximity to each other also showed high amounts of clustering. Of the 16 individual colonies (combined into 15) used in this study, there are 12 distinct branches in the phylogenetic network (Figure 3.6). Eight branches were a single colony, two branches contained two colonies each, (MR/KM and B5/B18) and one branch contained three colonies (DR/CM/EM). STRUCTURE at K=4, and multivariate analysis provides additional information for grouping colonies. Both analyses grouped colonies 1-3 though DAPC plots (Figure 3.4c and d) showed more separation between colonies 1-3 than is observed in STRUCTURE or the phylogenetic network. Colonies 1-3 and 15/TE were highly divergent genetic groups from the other colonies in each analysis. The colonies in the Gunnison Basin (7 branches) were part of a single population in STRUCTURE at K=4 and group together in DAPC and PCA plots. The greatest variation occurred with the three colonies in the Rio Grande watershed (Figure 3.7). Colony 4/RH was divergent in each analysis and showed little clustering with any other colony. Colony 5/ANT showed some association with colony 15/TE in the phylogenetic tree and with colony 6/SPGL in STRUCTURE and the PCA (Figures 3.3 and 3.4a&b). Colony 6/SPGL showed some clustering with the Gunnison colonies in the STRUCTURE and DAPC plots, notably the nearby colony 7/B9 (Figures 3.3 and 3.4 c & d).

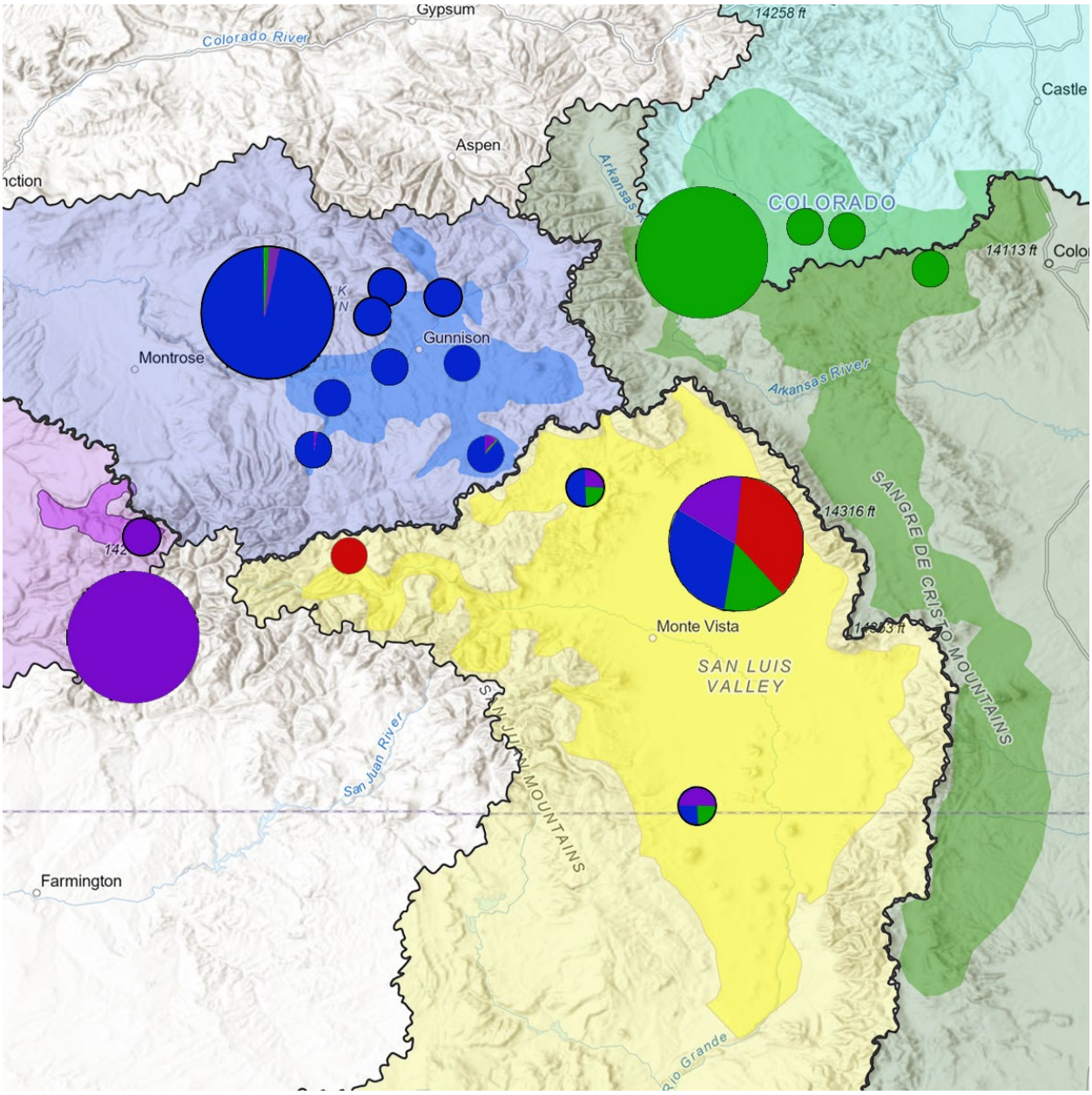


Figure 3.7: Map shows the sampled colonies and the approximate association to each of the genetic clusters from the K=4 STRUCTURE plot. Watershed areas are provided as background shading. Habitable regions of the 4 major regions occupied by *C.g. gunnisoni* are shown. Small and large circles show the proportion of assigned genetic structure found among all colonies.

Overview of genetic diversity:

Observed (Ho) and expected heterozygosity (H_E) range from 0.18 to 0.31 (Table 3.5). Measures of Ho are similar across all colonies, though there is greater variation in measures of expected heterozygosity. H_E ranges from a high of 0.30 in site 5/ANT to a low of 0.19 in 15/TE. The average amount of shared alleles within colonies ranges between 0.78-0.88 (Table 3.4). Measures of allelic richness (A_R) vary from 1.21-1.30. Colony 15/TE is notable as the colony with the lowest H_E (0.191) and allelic richness (1.21), and the highest proportion of shared alleles within a colony (0.88), indicating low levels of genetic diversity and adaptive potential (Table 3.4; Table 3.5). Colony 5/ANT, near the subspecies hybrid zone, has the highest values of H_O, H_E, and A_R, and the lowest proportion of shared alleles within the colony, showing high levels of diversity within this colony compared to other sampled sites.

Table 3.5: Genetic diversity measures for each sampled colony. The watershed the colony is in, the elevation of the site, and the number of individuals from each population are shown. Genetic diversity measures including the observed (H_O) and expected heterozygosity (H_E), and allelic richness (A_R) within each colony.

ID1	ID2	Watershed	Elevation (m)	Sample Size	H_O	H_E	A_R
DR	1	Arkansas	2672	7	0.267	0.26	1.25
CM	2	S. Platte	2641	11	0.286	0.26	1.26
EM	3	S. Platte	2615	12	0.26	0.25	1.25
RH	4	Rio Grande	3132	13	0.271	0.24	1.25
Ant	5	Rio Grande	2427	11	0.31	0.3	1.3
SPGL	6	Rio Grande	2674	8	0.298	0.28	1.28
B9	7	Gunnison	2817	14	0.269	0.27	1.27
B18	8	Gunnison	2392	12	0.258	0.27	1.27
HK	9	Gunnison	2895	13	0.256	0.25	1.25
MR	10	Gunnison	2502	9	0.254	0.27	1.26
KM	11	Gunnison	2647	11	0.257	0.27	1.27
Pow	12	Gunnison	2511	10	0.26	0.26	1.26
CR	13	Gunnison	2556	14	0.26	0.25	1.25
Gate	14	Gunnison	2503	6	0.247	0.22	1.23
TE	15	San Miguel	2663	11	0.275	0.19	1.21
Avg			2643	10.8	0.27	0.26	1.26

Evidence of unique genetic diversity:

There are no private alleles found in the VCF1 dataset. For VCF2, 54,844 additional alleles were kept in the VCF and 48,677 of them were private. Colony 15/TE had most of the private alleles, accounting for 34,786 of the additional SNPs alleles (Table 3.6). Colonies 3/RH, 5/ANT, and 14/GATE also stand out as having the next highest numbers of private alleles, each having between 3,400 and 3,700 private alleles, compared to with the next highest being just under 600. Private allele numbers are heavily influenced by rates of gene flow (lower private allele numbers if two sites have high amounts of gene flow) and sample size (more rare alleles are expected to be found with higher sample sizes), though colony 14/GATE had one of the higher private allele numbers despite having the fewest samples at six individuals. Private alleles are shown to increase with colony isolation (Figure 3.8), as distance and resistance (Chapter 2) predict the amount of unique diversity found within colonies.

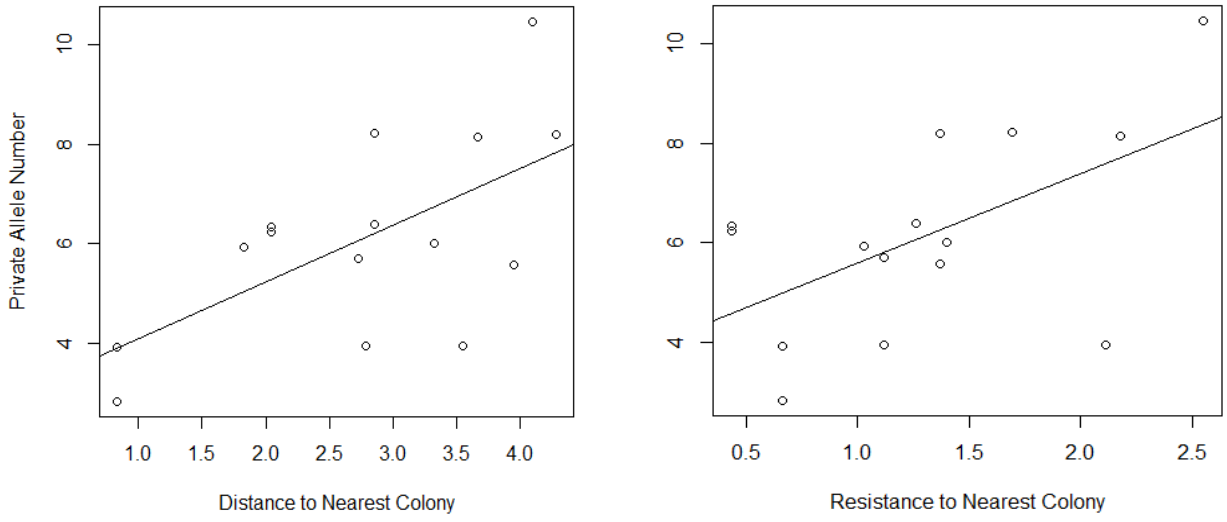


Figure 3.8: Relationship between the number of private alleles (log transformed) found in each colony and the distance (log of geographic distance (km) and log of resistance) between the colony and the nearest neighboring colony. A linear regression between log of geographic distance (km) and log private allele number is $r^2 = 0.38$ (p-value = 0.014). The relationship between log resistance and log private allele number is $r^2 = 0.32$ (p-value = 0.029).

Table 3.6: Genetic diversity measures and private allele number calculated from the VCF2 filtered dataset. For all colonies except -TE- Genetic diversity measures are like those of VCF1, though lower since more monomorphic alleles occur in each colony. Darker shading indicated higher private allele values. The private allele # for TE is so much higher, it is shaded as yellow to separate it from the other colonies and to highlight the relatively high private alleles numbers of some colonies compared to other colonies.

Pop ID 1	# Individuals	H_O	H_E	A_R	Private Allele #
Ant	11	0.247	0.229	1.23	3600
B09	14	0.184	0.18	1.18	406
B15	12	0.171	0.174	1.17	300
CM	11	0.193	0.169	1.17	563
CR	14	0.173	0.165	1.17	592
EM	12	0.192	0.166	1.17	514
Dr	6	0.181	0.154	1.17	52
Gate	6	0.186	0.156	1.16	3685
HK	13	0.167	0.16	1.16	376
KM	11	0.168	0.169	1.17	50
MR	8	0.166	0.169	1.17	17
Pow	10	0.171	0.165	1.17	51
RH	13	0.192	0.165	1.17	3422
SPGL	8	0.215	0.195	1.20	263
TE	11	0.288	0.191	1.20	34786

Prairie dog colony distribution may influence dispersal and overall connectivity. Colony locations in the Gunnisoni Basin (Figure 3.9) indicated that prairie dog colonies may occur in clusters. Figure 3.9 showed three large clusters of colonies that are within reasonable dispersal distances (under 6km) of each other. The largest cluster was to the east and north of the town of Gunnison, another occurred to the far east of the Gunnison Basin, and the third was to the north and to the east of Blue Mesa Reservoir. There are colonies located in non-overlapping polygons that are at least six km apart (from center to center) from other occupied colonies in the southeast (Cochetopa Park) and southwest portion of the Gunnison Basin. Lighter colors indicate likely paths prairie dogs would be expected to use when dispersing between colonies or colony clusters. The model in Figure 3.9 only accounts for distance, resistant features such as highways, towns, rivers, forests and topography may need to be considered as each will reduce expected dispersal capabilities (Chapter 2).

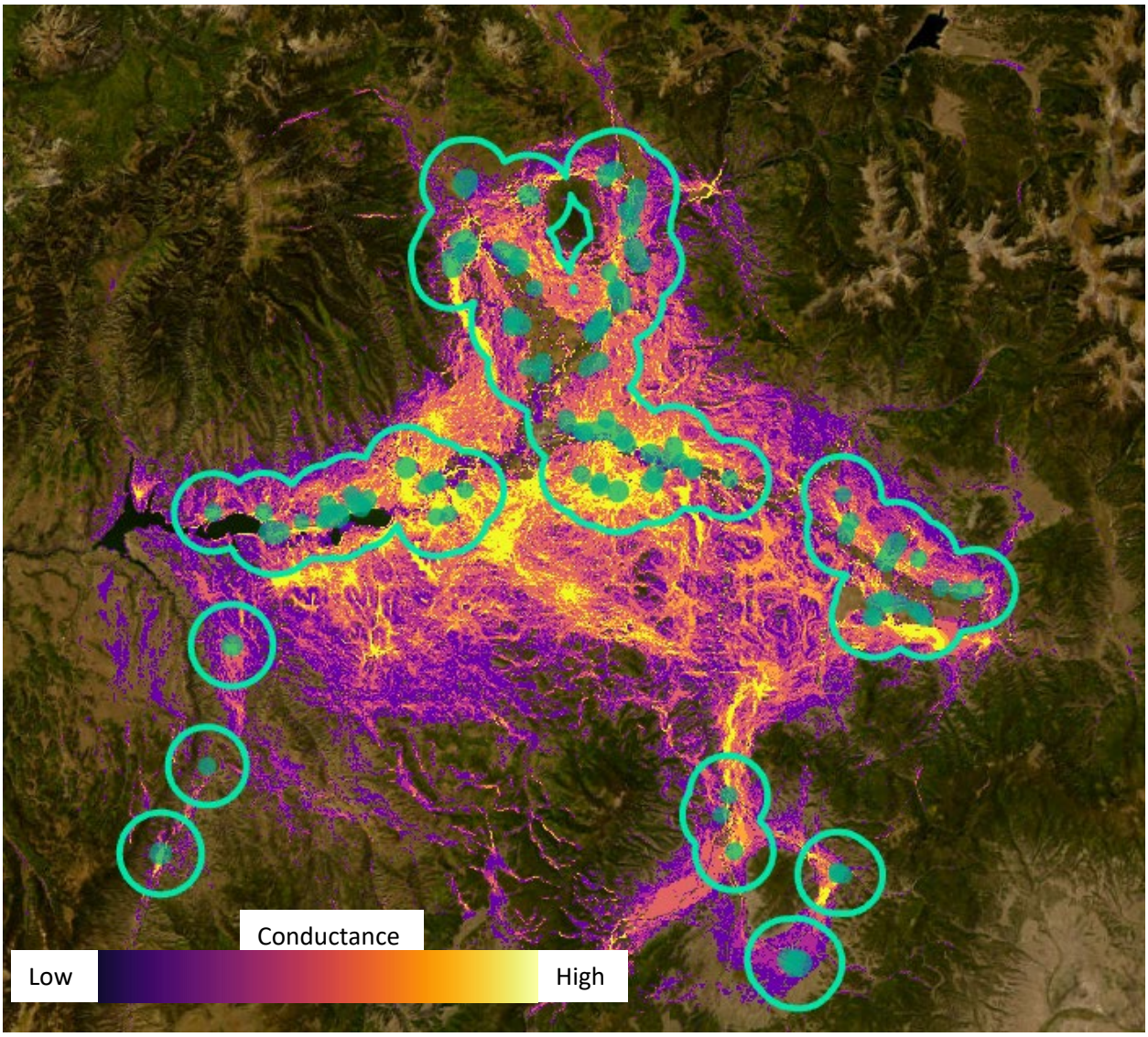


Figure 3.9: Colonies (cyan dots) from CPW (SAM data). Colonies were buffered by 3 km (cyan polygon) to represent possible dispersal distances across the topographic landscape of the Gunnison Basin. A connectivity model (see Chapter 2) shows likely animal movement paths across the landscape and may represent a historically connected metapopulation.

3.5 Discussion:

C.g. gunnisoni lives in the prairie and brush ecosystems in the southern Rocky Mountains. The subspecies range spans multiple large mountain valleys that are separated by high mountain ranges and forests that act as barriers to prairie dog movement. Despite these landscape features. In the last couple of centuries, populations have declined dramatically, and populations are estimated to be at only 1-2% of historic levels (Kotliar et al. 2006; Miller & Ceballos 1994). Currently, colonies are often found in low densities across much of the subspecies' range as eradication efforts, habitat loss and fragmentation, and disease has led to large declines in the numbers of individuals and reduced habitat occupancy. Colonies that were within population areas, like the Gunnison Basin, generally cluster more with each other than with colonies outside of population areas. There is evidence of IBD and IBR, indicating that dispersal is rare across distant colonies but common to nearby colonies (Figure 2.2, Chapter 2). I also observe low levels of genetic diversity and unique genetic diversity is harbored among populations that are distant or isolated from other populations (Figure 3.8).

Genetic clustering occurs among colonies:

The subspecies, *C.g. gunnisoni,* comprises multiple genetic groups. The genetic clustering among individuals is highly associated with individuals' geographic proximity and population structure shows evidence of being hierarchical. High genetic clustering occurs at the colony level as each colony is genetically distinct, except those in very close proximity to each other. Since females often stay within their national colonies, individuals within colonies are expected to be related (Kotliar et al. 2006; Figure 3.6). Genetic clustering weakens as the distance between colonies increases and when colonies are found across landscape barriers, such as mountain ranges. For *C.g. gunnisoni*, I consider the -colony- as the smallest population unit. Colonies that are in

close proximity to each other also show high amounts of genetic clustering, low genetic differentiation and higher proportions of shared alleles and may be considered part of a colony cluster (i.e., MR/KM, CM/EM; Tables 3.3 & 3.4).

Evidence of hierarchical population structure:

Most colonies are genetically distinct, but high genetic clustering occurs at different scales. Colonies within close proximity of each other show high amounts of genetic clustering as shown as individual branches on the phylogenetic network (Figure 3.6). Each branch could be one way to interpret a colony cluster. Close association of branches is patterned based on geographic proximity as neighboring branches are often next to the closest colony geographically. Colonies within population areas also show high intrapopulation area clustering and interpopulation area dissimilarity. Colonies located in large mountain valleys or basins, like South Park and the Gunnison Basin, are clustered more tightly to each other and more dissimilar to colonies outside the population area. STRUCTURE plot at K=4 provides an example of 4 distinct population groups, and one unrealized group (Figure 3.7). Three of the groups can be defined by the watersheds they are located in. Colony 15/TE is in the San Miguel, Colonies 7-14 are in the Gunnison, and Colonies 1-3 are in the South Platte/Arkansas watersheds. In Chapter 2 I concluded that the watershed boundary separating the South Platte and Arkansas watershed is not a significant barrier to prairie dog movement, so these regions can be thought of as a single population that I will refer to as South Park. These population areas are often separated by large mountain ranges that separate major watersheds and separate populations of *C.g. gunnisoni.* The one region where this observation is not apparent is among the three colonies in the Rio Grande population area.

Evidence of a large and complex metapopulation:

The colonies in the Rio Grande watershed have low amounts of genetic clustering. This population area consists of the large San Luis valley and the habitable portions in the San Juan and Sangre De Cristo Mountains. However, while there is little association between the Rio Grande colonies, there is also little association between the three Rio Grande colonies and colonies from other population areas. The low clustering between the Rio Grande Colonies could be partially due to IBD/IBR. The three colonies in the Rio Grande watershed are located at different ends of the population area, at distances that are much greater than colonies located in other watersheds (geographic distances of 72, 124 and 142 km between the three colonies, Figure 3.7). For comparison, these distances are greater than any pair of colonies within the Gunnison or Arkansas/South Plate (maximum of 64 km). Notably, despite being 124 km apart, colonies 5 and 6 have F_{ST} of 0.1, a value that is much lower than many F_{ST} values within the Gunnison basin with colonies that are much closer in geographic proximity. I would expect if more genetic samples were included from additional colonies in the San Luis Valley, a phylogenetic network would produce branches that would look similar to those that belong to the Gunnison colonies (blue) in Figure 3.6. Colonies 4/RH and 6/SPGL would be on the fringes of the tree, like colonies 7/B9 and 14/Gate are in the Gunnison Basin, and multiple other branches would populate the space in between and around colony 5/ANT.

High divergence and close ancestry:

The three colonies located in South Park and around Pikes Peak showed high genetic divergence from other colonies and high genetic similarity and close ancestral relationships (Figure 3.3 and 3.6). The high amount of clustering of colony 1/DR to the two colonies in South Park (2 & 3) is much closer than other colonies at distances greater than 20 km. The landscape between these two sites is also moderately resistant, with few dispersal paths linking these two regions (Figure 2.5). F_{ST}, and Jost's D values are much lower, and the proportion of shared alleles are much higher between 1/DR and 2/CM and 3/EM than colonies in the Gunnison Basin are at similar distances (Tables 2.3, 3.3 and 3.4). One explanation could be that gene flow in this region is, or was recently, much higher in the South Park area than in the Gunnison Basin. These three colonies represent populations in the northern portion of the habitable areas in the South Platte and Arkansas watersheds. Additional research is needed to confidently determine the genetic and demographic relationships with colonies that span the other habitat areas within the South Platte and Arkansas watersheds and if they cluster with the three colonies located in and around South Park.

The population with the highest divergence from other populations is colony 15/TE in the San Miguel watershed. This colony is separated from all other colonies in each analysis (Figures 3.3, 3.4, 3.6) and is on a single long branch in the phylogenetic network (Figure 3.6). This divergence in genetic compositions could partially be due to high geographic separation and potential long evolutionary isolation from other *C.g. gunnisoni* populations. The prairie dogs near Telluride CO are the most unique group within *C.g. gunnisoni*, with high amounts of genetic divergence from other colonies as well as harboring many alleles unique to this population. I expect this population has been isolated from other *C.g. gunnisoni* populations longer than other

populations I sampled. It is also likely that some alleles found in the Telluride population are due to gene flow between *C.g. gunnisoni* and nearby *C.g. zuniensis* population (Figure 3.10; Table 3.3 & 3.4). Sackett et al. 2014, observed that these populations showed high genetic distinctiveness from both *C.g. gunnisoni* and *C.g. zuniensis* using microsatellite genetic markers, although mitochondrial sequence data matched *C.g. gunnisoni* haplotypes.

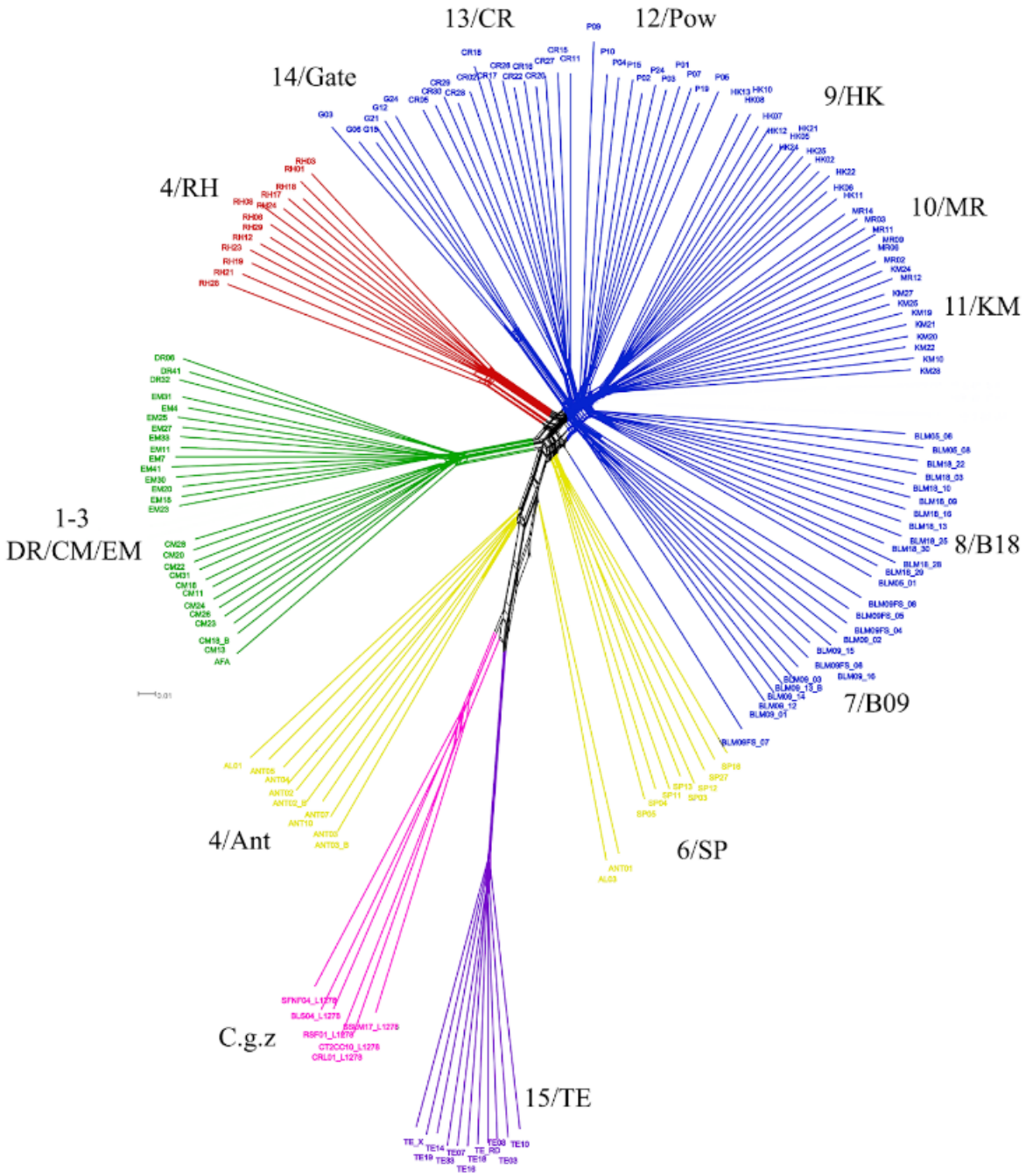


Figure 3.10: A phylogenetic network including some individuals from C.g. zuniensis (pink). There is an association with both 15/TE and 4/ANT colonies with the *C.g. zuniensis* individuals. Both colonies are near the contact zones of the subspecies and the long branches connecting these three groups together suggest a tangible, though distant relationship between them.

Colony distribution in the Gunnison Basin:

The Gunnison Basin provides a large habitat area consisting mostly of grassland/sagebrush ecosystem and rolling hills and has the most sampled colonies. The core region of the Gunnison basin is sagebrush habitat that covers rolling hills and ridges. The Tomichi Creek runs from the east side of the basin until it meets the Gunnison River near the town of Gunnison. North of the town of Gunnison, the Taylor River, East River, and Ohio Creek flow south and finally converge north of the town of Gunnison. The Basin is divided north to south by Tomichi Creek, Gunnison River, and Blue Mesa Reservoir. Multiple other drainages and canyons divide the landscape including both Cochetopa Creek and the Lake Fork River to the south.

To the northeast of the Gunnison Basin is Taylor Park, where prairie dogs may have once inhabited, but no prairie dog colonies are known to occur now (Seglund and Schnurr 2010). To the southwest of the Gunnison basin is another small basin called Cochetopa park, where some prairie dog colonies exist. The Cochetopa hills, a low-lying mountain range, separates the Gunnison watershed from the Rio Grande. Colony 7/B9 is in Cochetopa Park and colony 6/SPGL is located nearby across the Cochetopa Hills in the Rio Grande watershed. The Cochetopa Hills were a region that may or may have allowed gene flow to occur between the Gunnison and Rio Grande watersheds. Colonies 6/SPGL and 7/B9 showed a close association in PCA and DAPC plots (Figure 3.4) and phylogenetic networks (Figure 3.6), suggesting gene flow occurred between colonies separated by the Cochetopa Hills.

In the Gunnison Basin there are multiple hypotheses for how colonies can be grouped. The phylogenetic network (Figure 3.6 and Figure 3.10) indicated that most colonies, except those that are neighboring (approximately 5 km or less), could be considered a genetic cluster. STRUCTURE

plots proposed that all colonies in the Gunnison Basin are part of a single population (Figure 3.3). Further, information about colony locations and expectations of dispersal capabilities showed that multiple clusters of colonies occur in the basin, each separated from each other by large areas of uninhabited space (Figure 3.9). I expect that the complex patterns shown in the Gunnison Basin will occur in each of the population areas, though each will be unique based on the area's habitat and distribution of colonies. For example, genetic differentiation may be greater at similar distances within the Gunnison Basin than it is at similar distances in areas like South Park or the San Luis Valley.

Genetic structure, connectivity, and population distributions can inform managers about past genetic relationships and current population dynamics for the purpose of developing management units for conservation purposes. Deciding which management goals and conservation strategies that will be implemented may influence how MUs are designated and for what purpose. Additional research that further evaluates the unsampled regions in the *C.g. gunnisoni* range, investigating the evolutionary processes occurring at subspecies contact zones, and identifying adaptive differences between populations would be recommended to contribute additional genetic information for the purpose of delineating MUs for *C.g. gunnisoni* (Funk et al. 2012).

Small populations and genetic diversity

Plague, fragmentation of populations, and reductions in connectivity can have effects on the genetic composition of colonies and metapopulations. Allele diversity, unlike heterozygosity, is highly susceptible to dramatic decreases in population numbers (Allendorf et al. 2022). If populations remain small and gene flow is low, genetic drift can further erode heterozygosity and allelic diversity, potentially leading to increased chances of inbreeding and its negative effects on population fitness (Reed and Frankham 2003; Noss 2004; Frankham 2014; Chen et al. 2016;

Fitzpatrick et al. 2020; Tian et al. 2021). Recent declines in population size are expected to reduce allelic richness. If populations remain small and gene flow is absent, genetic drift will act on reducing heterozygosity. I observed that allelic richness and heterozygosity is low among individual colonies. This could indicate that recent population declines have reduced the allelic diversity within colonies. Two colonies that stand out as having exceptionally lower heterozygosity and allelic richness are 14/Gate and 15/TE. The high isolation of each of these colonies and low gene flow could allow for genetic drift to act on these populations and erode genetic diversity.

Inferences gained from genetic structure were limited by the location of samples across the range of *C.g. gunnisoni*. Only the Gunnison Basin was well represented. A more complete picture could be developed if additional colonies are sampled in the other population areas. Another consideration is the use of low coverage whole genome sequencing. These sequencing methods provide genetic information across the entire genome, but the depth per site is low. This leads to high amounts of missing alleles and genotype uncertainty.

Fragmented and isolated populations should be managed in a way that prompts connectivity and preserves colony occupancy and genetic diversity:

Historically, Gunnison's prairie dogs were much more abundant than they are today (Seglund et al. 2005: USFW 2008; Ecke and Johnson 1952). With human settlement of the region, habitat loss and eradication campaigns led to reductions in prairie dog populations on lands used for farming and ranching. Despite the negative impact of these activities, it was not until the introduction of plague to these ecosystems that massive declines were recorded in prairie dog numbers, and plague continues to represent the primary threat to colony persistence (Cully et al.

1997; Kotliar et al. 2006). Managing plague is an important part of conserving prairie dogs and reducing the frequency of colony extirpation caused by the disease.

Unique variation is harbored in isolated colonies:

I found that unique genetic variation is harbored in colonies that are isolated from other populations (Figure 3.8). To conserve the greatest amount of existing genetic variation in *C.g. gunnisoni*, these -isolated- colonies could warrant increased conservation attention. If these colonies are lost, a localized extinction could occur, removing prairie dogs from part of the landscape while also losing allelic variation in the subspecies. Plague is the primary concern for colony extirpation and plague management has been used to reduce the risk of colony extirpation from sylvatic plague on Gunnison's prairie dog colonies since 2010 (Seglund et al. 2022; USFWS 2013; Rocke et al. 2017; Tripp et al. 2017). Plague management is an important strategy for maintaining colony occupancy and preserving steppingstone colonies that facilitate gene flow across the landscape. Despite its conservation benefits, plague management may not be sufficient to address pertinent issues such as restoring population connectivity, genetic diversity, increasing effective population size, and restoring colony occupancy in at-risk areas.

Low gene flow across population areas:

Geographically separated colonies and clusters of colonies may be demographically isolated (Figure 3.9). Prairie dogs may not be able to disperse across large distances of uninhabited space, limiting gene flow and connectivity among spatially separated colonies. When extreme isolation and inbreeding occur, genetic rescue -increasing the genetic diversity of a population through the introduction of individuals from another population- has been shown to increase genetic diversity of a population with low genetic diversity (Keepers et al. 2018; Fitzpatrick et al.

2016). Dramatic increases in genetic diversity and individual fitness can be obtained with the introduction of just a few individuals with differing genetic compositions into a population's gene pool and can impact a population's fitness (Frankham 2014; Kronenberger et al. 2017; Fitzpatrick et al. 2020). Gene flow between colony clusters may provide large increases in genetic variation. Assisted migration can therefore be an alternative management strategy when dispersal is unable to overcome the effects of genetic drift.

However, translocations of individuals can be risky. Outbreeding depression, the spread of disease, disruption of social behaviors, immigrants outcompeting resident individuals, and low survival due to individuals being poorly adapted to their new habitat are some concerns when moving individuals to new populations (Hess 1994; Bright & Morris 1994; Martin et al. 2012). Moving individuals near subspecies ranges should be avoided if managers want to reduce genetic mixing of subspecies. The directional movement of alleles from *C.g. zuniensis* to *C.g. gunnisoni* could mean that *C.g. zuniensis* may outcompete *C.g. gunnisoni* (Martin et al. 2012), though as climate change occurs, habitats each subspecies is adapted to may be shifting north and up in elevation (Garroway et al. 2011). Moving individuals from the subspecies contact zones should be avoided until more is known about the dynamics of hybridization between the subspecies. Until research evaluates the responses to translocation efforts, managers should be cautious about where prairie dogs are moved. In most cases, I suggest performing translocations between colonies that are in the same population area between colonies at occur in similar habitat types and elevations. In some cases, such as with the sparsely populated region around Telluride or with colonies at extreme elevations, the best source colonies for a translocation may be those in different population areas. Despite some concerns, management strategies that transfer individuals between

geographically separate colonies could be helpful in increasing the genetic diversity of populations and increasing the ability for Gunnison's prairie dogs to adapt to a changing environment.

Conclusion:

I used low coverage whole genome sequencing to characterize patterns of genetic structure and estimate genetic diversity in a species of conservation concern. I found that *C.g. gunnisoni* is composed of multiple genetic clusters with limited evidence of gene flow between clusters. Gene flow is expected to be higher with less geographic distance and more habitat continuity between colonies; conversely gene flow is expected to be restricted between more geographically distant localities and among colonies inhabiting patches embedded in a landscape with limited evidence of corridors with suitable habitat. Additionally, genetic diversity within colonies is low; this condition predicts many areas may experience localized extirpations and a continued trend of declining numbers of animals unless explicit conservation actions are implemented that are designed to counter mutational meltdown (Lande 1988; Lynch et al. 1995; Frankham 2005). Furthermore, plague remains a constant threat, expanding plague management to increase the number of managed colonies and distribute protection across the species range will benefit the persistence of colonies and occupancy across the subspecies range.

CHAPTER 4

POPULATION ECOLOGY OF THE NORTHERN SPOTTED OWL:

A VIRTUAL LESSON

4.1 Abstract

The mathematical modeling of populations utilizing field-collected demographic data is an important component of lab curricula in a variety of undergraduate biology lab classes. During the global pandemic brought about by the SARS-COV-2 virus in 2020, I and a group of other teaching assistants successfully converted an in-person lab on demographic population modeling to a lesson that could be offered remotely. We used a Google Earth Web Project to simulate a population study of the Northern Spotted Owl. In the simulation, students navigated transects and collected both demographic and mark-recapture data based on images of Northern Spotted Owls seen along each of four different transects. In addition, students monitored two simulated GPS-tagged owls. After gathering data students used the data to determine population size using the mark-recapture method, they derived a life table, and calculated the net reproductive rate. Students used this combined information to assess the current management plan for the population studied and ended the lesson with a literature search on the associated population trends of the Barred Owl in relation to the Northern Spotted Owl and logging practices in old-growth forests. In this paper I outline the remote learning population ecology.

4.2 Introduction and Background Information:

The SARS-COV-2 pandemic forced many in-person, lab, and field-based lessons to become virtual and remote (Garcia-Vedrenne et al. 2020, Morrison et al. 2021). While this change was specifically caused by the pandemic, the need for remote-style lessons continues, not only to be available when the next pandemic hits, but also to give students who miss class for whatever reason an opportunity to complete the lesson remotely. I and a small team of other graduate teaching assistants responded to this need by revising curricula in ways that kept key learning goals intact, retained interest in relevant biological scenarios, and engaged students. A popular laboratory-based lesson that focused on the population ecology of Northern Spotted Owls was adapted to be fully remote. The lesson emphasized data collection, manipulation, and analysis; enabled practice of key quantitative skills; and emphasized reasoning and constructing evidence-based claims. The lesson utilized a virtual platform, Google Earth Web, to allow students to interact and engage with simulated data. Importantly, the lesson framework can be easily adapted for other species and locations depending on the interests of instructors and students living in different parts of the world.

The challenge for any transition to a virtual platform is to effectively incorporate the goals of an in-person class into the virtual classroom. For undergraduate biology labs, important goals are to facilitate manipulative skills, quantitative skills, and reasoning skills associated with research and potential jobs in a variety of subdisciplines within the field (AAAS 2009). One such subdiscipline of biology is population ecology and within the subdiscipline is the demographic and mathematical modeling of populations including growth, dynamics, control, and sustainability. This focus is important not only because it is a major source of job and research opportunities on such items as endangered and threatened species (Santana et al. 2020, Margalida et al. 2020),

invasive species (Jelbert et al. 2019), human population growth (Hilde et al. 2020), and many others; but it also is important for building quantitative skills important for achieving quantitative literacy which is essential to achieving biological and science literacy (see Baumgartner et al. 2015). In addition, this focus has wide-ranging unexpected applications such as understanding and modeling the growth and spread of disease like that of SARS-COV-2 (Russo et al. 2020).

Intended Audience:

This lesson was originally designed as a single class lab experience within the lab component of an introductory undergraduate General Biology course, a one-credit hour lab class associated with a three-credit-hour lecture. The original audience was a lab class enrolled by approximately nine hundred students divided into sixty sections and taught by twenty graduate student teaching assistants (GTAs). The lesson can be adapted for other audiences and can be effectively utilized in both a lab and a lecture setting as well as classes ranging from introductory level biology to ecology, to environmental science. This lesson provides a framework that allows educators to adapt the biological system to any desired species at any location in the world.

Required Learning Time

The lesson plan is designed to be completed within a two-hour time period. No prior learning before class is needed. Data collection, analysis, and assessment is expected to be completed by most students within two hours, though the ability for this to be a self-guided lesson can allow students to work at their own pace.

Prerequisite Student Knowledge

This lesson is designed to function on its own and students do not need any prerequisites to successfully complete this lesson. The lesson incorporates mathematical functions with algebraic expressions so a background in algebra is helpful but is not required. Likewise, an understanding of science-process skills, and a basic understanding of how to perform a literature search will also be helpful. Introductory material on population biology is provided at the beginning of the lesson. If desired, this lesson can be integrated into a class lecture or adjusted to the teacher's preferences.

Prerequisite Teacher Knowledge

Instructors should be comfortable with concepts associated with population biology such as demography (births, deaths, age structure), telemetry, life tables, mark-recapture methods, the use of transects, and population growth rates. Additionally, teachers will need to be familiar with the online platform Google Earth Web and be able to accurately sex and age spotted owls in provided photos. Teachers are expected to spend 1-2 hours reviewing the lesson on their own prior to teaching the module. Going through the lesson and associated materials is expected to provide enough training for teachers to answer student questions and successfully run the lesson.

Scientific Teaching Themes

Active Learning

Students will engage in active learning by interacting with a Google Earth Web Project to collect data. Students will make "observations" by examining photos of owls that were taken along simulated transects and record their observations onto a data sheet. The students will use the results of these observations to calculate demographic measures. The results of these measures will be used to address if the management program is succeeding based on student calculations.

The lesson exercise:

During the lesson, students will be required to fill out multiple tables. Table 4.1 is used to record the observations made by the student from each of the Northern Spotted Owl photographs. This includes the age, sex, whether it was banded, and the number of fledglings (Table 4.1). The students will then use the observations from Table 4.1 to summarize the number of alive and dead owls that were found in each sex and age class (Table 4.2). Finally, the age class data will be used to complete a life table (Table 4.3).

Table 4.1: Record observation of Northern Spotted Owls. The first 5 rows of the table are given.

Sex	Alive or Dead	Banded (Y or N)	Age Class	# Of Fledglings

Table 4.2: Owls by age class: use this table to fill out how many males and females of each age class were observed during the observation period.

Age Class	Males Alive	Males Dead	Females Alive	Females Dead
0-1				
1-2				
2-8				
8-13				
13-18				

Table 4.3: Life Table: Use the data in Table 4.2 to complete this life table. Calculate the survival probability within an age class (sx); survival probability from the beginning of life to the end of an age class (lx); the fecundity of individuals within a specific age class (bx); and the net reproductive rate (R0).

Age Class	s_x (females)	l_x	b_x	$l_x b_x$
0-1				
1-2				
2-8				
8-13				
13-18				

Data analysis: The information from the life table will be used to estimate the number of owls of each age class that are in the population. Students will also calculate the net replacement rate using these data.

Post Module: Students will be asked to evaluate their results and provide evidence to support their statement on whether the management program is working or not.

4.3 Lesson Plan:

The Population Ecology module was used in the 2020 and 2021 spring semesters. The lesson duration was 110 minutes, was remote, and was completed by students without direct intervention by the teacher. If desired, this lesson can be conducted in class or in groups without any modifications. Most of the variation in time required for completing the lesson was due to differences among students in their time on the end-of-lesson assessment questions; thus, students who require additional time are best served if the lesson time can be up to three hours or are expected to complete questions as homework. Since the lesson was provided remotely and asynchronously, the lesson was available for a week and without time constraints to allow students to work at their own pace. The results and answers to the assessment questions were submitted at the end of the week.

Instructor Preparation

The material provided within the lesson is expected to provide the necessary preparation for instructors. Instructors should make sure they complete the lesson on their own and understand how to use the Google Earth Web interface. It will be necessary to understand the basic concepts of population biology, demographics, and the calculations to complete life tables. Additionally, teachers need to be able to correctly identify the owls from photos. If teachers complete the lesson and the results from the life tables are accurate, this will demonstrate sufficient training to help students if problems arise. Teachers can provide additional introductory materials if they choose to do so.

Student Preparation

In this lesson, it is assumed that students are not fluent in any of the concepts related to this lesson. The approach did not require students to have completed any pre-lesson work. If desired, additional introductory materials, readings, or pre-assessment questions could be assigned to students prior to the lesson.

Background and Introduction:

Human perturbation of habitats has led to a potentially prominent threat to modern ecosystems by the invasion of species. Non-native invasive species that are introduced into a new habitat are typically used to demonstrate this threat because species within ecosystems are adapted to that ecosystem based on millions of years of interactions. When a new species is introduced, if conditions are right, the species has enormous potential to proliferate within the new ecosystem. But what about **native invasions** due to habitat alterations, such as those caused by climate change or local human activities? Can alterations in habitat caused by people favor one native species over another? This is a question that can be addressed by population biologists and will be the topic of this lesson.

Prior to recent human activities, the Pacific Northwest (Northern California, Oregon, and Washington) was dominated by what is known as late successional or old growth forests. Old growth forests are characterized by trees greater than two hundred years of age, which have a high number of snags or broken tops, which shed needles and create a relatively sparse understory. Many animals have been adapted to thrive in old growth forests such as northern flying squirrels. Northern Spotted Owls have many adaptations for old growth forests. One such adaptation is related to nest building; they prefer to nest high up in the snags of large old-growth trees and they build their nests accordingly. Periodically in the past, fires and other natural disturbances would wipe out vast areas of old growth forest and create early successional patches within the old growth

forest that would favor animals with different adaptations such as Barred Owls. Over the last 200 years, human disturbance through logging has changed the vast expanses of old growth forests and has changed much of the forest habitat in the Pacific Northwest to new growth forests (< 200 years). In the 1980's population biologists noticed populations of Northern Spotted Owls were declining rapidly and in 1990, the Northern Spotted Owl was designated as threatened under the endangered species act. Since 1990 a recovery plan has been enacted and an attempt to preserve critical Northern Spotted Owl habitat was carried out. In this lesson, you will act like a population biologist and gather information on a representative current Northern Spotted Owl population. Before you begin your study, you will need background information.

Population Growth and Demography

The rate of growth of a population is determined by four factors: **births**, **deaths**, **immigrations** (animals entering the population from another population), and **emigrations** (animals leaving the population). In many cases, and for cases in this exercise, immigration and emigration account for little change compared with that of births and deaths. Therefore, the lesson will concentrate on the influences of births and deaths on the size and growth of populations.

Birth rates and death rates may depend upon the age structure within the population. For instance, grizzly bears can live more than 20 years, they typically do not start reproducing until an age of five, and their mortality rate is high for the first 4 years of life, decreases in middle-aged bears, and increases after an age of 15 (Knight and Eberhardt 1985). Therefore, the rate of growth of a population of grizzly bears would depend on the relative frequencies of young individuals, middle-aged individuals, and old individuals in the population. The study of age-specific and other statistical factors influencing the size of a population is called **demography**. Population demographics are determined through research and organized into **life tables**. Two types of life

tables are **cohort life tables** and **static life tables**. A **cohort** is a group of individuals born at the same time or during the same season. A cohort life table is developed by marking a cohort at birth, following them throughout their lives and estimating age-specific **fecundity** (reproduction) and survival rates. A cohort life table is developed through a longitudinal study and may take many years. For instance, to develop a cohort life table for grizzly bears it may take longer than 20 years of research (Knight and Eberhardt 1985). In contrast, a static life table is developed by aging and marking a randomized sample from the entire population all at the same time, then following them for a short period of time to determine age-specific fecundity and survival rates. Age-specific fecundity of male animals is often difficult to determine especially when the mating system is not monogamous. Therefore, in the life table, you will examine age-specific fecundity and mortality of females only. The following symbols are important to know for evaluating life tables (See Noon and Biles 1990).

***x* = age class.** An age class is a group of individuals in the population that are a certain age (0, 1, 2, 3, ... years old), or group of ages (0-4, 4-8, 8-12 ... years old). Age classes are usually determined by the ability of a researcher to distinguish the age of the organism, the size of the population, and the generation time of the organism.

s_x = survival in age class. The probability an individual survives from the beginning of an age class to the end of the age class and is calculated by the equation:

Alive in age class / (# alive in age class + # dead in age class)

***l_x* = survival to age class x.** The probability an individual survives from the beginning of their life to age class *x*. The survival to age class x can be determined by multiplying values of s_x for all age classes $\leq x$. For example, if you wanted to determine l_3, you would multiply s_1, s_2, and s_3.

***b_x* = age-specific fecundity.** The number of female offspring produced by a female during age class *x*. Under situations where you cannot determine the sex of the offspring you can assume that the sex ratio of males to females is equal. You can determine the age-specific fecundity rates from the equation:

b_x = 0.5(# of offspring in age class / # of females in age class)

***R_o* = net reproductive rate.** The number of female progeny produced by a female during its lifetime. The net reproductive rate can be determined by summing the products of l_x and b_x in each age class ($l_1b_1 + l_2b_2 + l_3b_3$ ...). This value indicates whether each individual can replace itself in its lifetime. If $R_o = 1$ the individual replaces itself exactly. If $R_o < 1$ then the individual does not replace itself, and thus, the population is decreasing. If $R_o > 1$ then the individual over-replaces itself, and thus, the population is increasing. If R_o is 0.5 then the individual is only replacing one-half of itself over its life. Note that R_o is an average value for all individuals in the population.

Mark-Recapture Method of Estimating Population Size

One method of estimating total population size from a sample is the mark-recapture method. The mark-recapture method involves capturing a certain subset of the total population, marking them in some way that they can be recognized in the future, releasing them back into the population, then capturing another subset of the population. For this lesson you will use a modified

mark-recapture method and use a sight-resight method. From knowing the total number of individuals marked, and the number of recaptures during the second capturing bout, an estimate of the total population size (*N*) can be made from the following equation.

N = ((total # in pop. with a band) (# of living owls seen)) / # of owls seen with a band

For this lesson, suppose that the total # in the population with a band is thirty. In order for the mark-recapture method to be valid, the probability of capturing any individual in the population must equal the probability of capturing any other individual. When using traps, animals cannot become trap-shy or trap-happy. When using a mark-resight method, the re-sighting trips must be randomized rather than on the same pathways over and over.

GIS and GPS

Global positioning system (GPS) data is important for recording positions in the world. These data can then be used in geographic information systems (GIS) and global visualization tools (such as Google Earth, ArcGIS Explorer, and QGIS). This integration of geospatial technology of research, industrial, or commercial fields. For biologists, GIS and GPS have become essential for most researchers and managers as a way to plan projects, track individuals, or analyze data from environmental systems. Incorporating GIS technologies, GPS data, and visualization platforms allows biologists to better understand the environment and make responsible environmental decisions (Carrarra & Fausto, 1995; Heit, Shortried, & Parker, 1991; National Research Council, 2006). Google Earth provides an easy-to-use interface that allows anyone to easily explore and visualize spatial data in a world model.

Student Procedures

Students are provided a link to the Google Earth Web Project (https://earth.google.com/web/@40.01152752,-105.268388,1614.00740078a,2057.55002494d,35y,0h,0t,0r/data=MicKJQojCiExVDU0blhDc013elRGNGdRUU4xb3ZZckRnZkp0NW9MTEk6AwoBMA?authuser=0) "Population Ecology." and guided through their virtual mark-recapture module. Clicking on the "Present" button opens the Google Earth project presentation. A brief overview of wildlife tracking with radio telemetry and GPS tags provides background on how wildlife biologists can track wildlife. The use of GPS tags provides an opportunity to incorporate how GIS software, like Google Earth, is utilized as a tool for monitoring populations on the landscape. Two GPS-tagged owls were provided as examples for how wildlife can be tracked with GPS data. The tracks from these GPS-tagged owls are drawn onto Google Earth, allowing students to visualize the paths owls take and identify locations where owls spend time. Points where owls commonly visit or occupy for extended periods are likely either nest sites or perches. Photos of each perch are shown, and one contains our tagged owl next to a fledgling. Students then utilize the owl identification sheet (provided in the Student Handout; Contact Sean Streich for updated supplementary information including the Owl Identification sheet and the Student and Teacher handouts) to help determine the sex and age of the owl. Students finally record the following information about each owl encountered in Table 4.1, the sex, the age, whether the owl is alive or dead, how many fledglings the owl has, and whether the owl is banded. Being banded means the owl has been captured previously and was marked with a color-coded band around the leg.

After another example of a GPS transect, an example of a deceased owl is provided. In this case, a Northern Spotted Owl has been observed in the talons of a great horned owl. Students

record the information on the deceased owl in Table 4.1 as well. Students continue from the two GPS-tracked owls to three transects with images of owls along the transects. Students record information on each owl they encounter along the transects in Table 4.1.

When students finish the owl identification, they are finished with Google Earth Web. They can apply the information in Table 4.1 to the mark-recapture method and estimate the overall size of the population. In addition, in Table 4.2, students are asked to quantitatively distill the data in Table 4.1 by compiling totals of living and dead owls observed by sex and age class, and the associated fledglings for each female. Information in Table 4.2 is applied to completing life-table calculations (Table 4.3). In the life-table students calculate several parameters for each age class: survival probability within an age class (sx); survival probability from the beginning of life to the end of an age class (lx); the fecundity of individuals within a specific age class (bx); and the net reproductive rate (R0).

Once Table 4.3 is completed, the students are finally asked to assess the status of the population studied and relate the assessment to the implemented management plan (i.e. what is currently happening to the population; from the life table is there any demographic information indicating vulnerabilities in the success of the population that the management plan may need to address; and is it even possible to reverse any trends in the population?).

Finally, the last step in the assessment is for the students to extend the findings of this research to another research study. Since the final learning goal pertains to helping students go beyond the species level interaction and think more along the lines of community level interactions, students are asked to find a paper in a peer-reviewed journal that describes original research on the relationship between the Spotted Owl and Barred Owl, provide a brief synopsis on that article and provide a full, APA-format citation for their paper.

Teaching Discussion

Teaching labs during the Covid-19 pandemic revealed the critical need to have more high-quality lab experiences available that can be taught remotely. Outside the pandemic, remote lessons can also have value. For example, when students are sick or miss a day of class, a remote lesson can be implemented so the student can make up the material on their own. We adapted a field-based simulation of a population ecology lesson to be conducted through a GIS platform. While some aspects of field-based methods were lost, such as using radio transmitters and conducting wildlife observations with binoculars, students were introduced to methods of using GIS and GPS data in a population ecology study and visualizing the data on Google Earth web platform. Also, this lesson can be very adaptable, with any species of interest being the focus of the lesson. Data can be simulated or obtained from real world monitoring results. In addition, this lesson can be conducted from any computer platform from anywhere in the world and designed around locally relevant scenarios or focus on a charismatic species from anywhere in the world. Imagine gathering data on the populations of the Black Rhinoceros in Africa. All that is needed is an internet connection and a browser. While this lesson was developed to be completely independent from teachers, it is not required. Teachers can edit the amount of introductory material, ask different questions, or make the assignment group based. The lesson outlined in this paper provides the framework for teachers to choose a biological system that will work best for their students.

4.4 Conclusion

The ability to visualize and track how the earth changes has drastically changed for scientists and nonscientists. Now, many people use mapping services daily for travel. Google Earth

provides imagery of the world that is continuously updated and accessible to anyone with a computer and internet connection. Recently, news agencies across the world would provide continuously updated visualizations of how the SARS-CoV-2/COVID-19 pandemic spread across the world, created using information from GIS and web-mapping technologies (Zhou et al. 2020; Rosenkrantz et al. 2020). Our society has become used to GIS technologies, from navigation like google maps to fitness trackers used with watches. Integrating these technologies that are now very familiar to students into biological thinking can be valuable for communicating and teaching science. Models that provide geospatial information about the world can be applied to many aspects of biology and can be valuable for the next generation of critical thinkers to progress research and society.

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