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Assessing changes in connectivity and abundance through time for fisher in the southern Sierra Nevada

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ASSESSING CHANGES IN CONNECTIVITY AND ABUNDANCE THROUGH TIME FOR FISHER IN THE SOUTHERN SIERRA NEVADA

By

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ABSTRACT

Small populations are at increased risk of extinction due to their vulnerability to stochastic events. The population of fisher (*Pekania pennanti, formerly Martes pennanti)* in the southern Sierra Nevada Mountains of California is small and completely genetically isolated. My dissertation research investigates the timing and cause of this population's isolation, the degree of genetic subdivision within the population, the landscape features shaping gene flow, and the detection of population declines.

I detected a 90% decline in effective population size and dated the time of decline to over a thousand years ago. Analyzing historical and contemporary genetic samples, I also found a recent bottleneck signal in the northern portion of the southern Sierra Nevada, indicating the southernmost tip of these mountains may have acted as a refugium for fisher in the late 19th century. I conclude that this population became isolated pre-European settlement, and that portions of the southern Sierra Nevada subsequently experienced another more recent bottleneck post-European settlement.

I found that the southern Sierra Nevada fisher population is not highly genetically subdivided as previously thought. This population follows a pattern of isolation by distance with additional structuring that corresponds to geographic features and management boundaries. It can be characterized as having areas that are resistant to gene flow but without major barriers. I show that both sex-biased dispersal and spatial landscape heterogeneity can affect the determination of what landscape features structure gene flow, and that the landscape features influencing gene flow are different for each sex and within different geographic regions.

Using a spatially-based simulation approach, I investigated the power of the Sierra Nevada fisher monitoring program to detect population trend, and illustrate the relationship between occupancy and abundance in this population. I show that a simulated 43% decline in abundance over an 8-year period only resulted in a 23% decline in occupancy. I also found that increasing the effective sampling area, implementing biennial instead of annual sampling, and increasing the type I error rate all increase statistical power to detect trend. Overall this research provides a better understanding of the historical and contemporary connectivity of this population and our ability to monitor population trends over time that will contribute to the conservation of fisher populations in the future.

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Chapter 1: Introduction and Overview

Small populations are at increased risk of extinction due to their vulnerability to stochasticity in environmental factors, demographics, and genetics (Gilpin & Soule 1986). Connectivity to other populations is one way in which small populations can increase their probability of long-term persistence through the influx of new individuals that increases both population size and genetic diversity (Hanski 1999). Therefore, conservation concerns are particularly acute for populations that are isolated as well as small. Effective conservation strategies for such populations require accurate information about the historical and current distribution, abundance, and demography of the population. The extent to which these population characteristics have changed over time are important criteria in determining species' or populations' conservation status (ESA 1973, IUCN 2001). However, detailed studies of populations over time are often logistically difficult and costly. This is especially true for rare species occurring across large geographic areas.

The population of fisher (*Pekania pennanti* [Kopefli *et al*. 2008, Sato *et al.* 2012], formerly *Martes pennanti* [Erxleben 1777]) in the southern Sierra Nevada mountains of California is small (<300 adults, Spencer *et al*. 2011), and completely geographically and genetically isolated (Wisely *et al.* 2004; Zielinski *et al.* 2005; Knaus *et al.* 2011). It is currently a candidate for listing as part of the West Coast distinct population segment under the federal Endangered Species Act (U.S. Department of the Interior, Fish and Wildlife Service 2004). Previous research found extreme genetic subdivision within the population (Wisely *et al.* 2004). Detection of this strong subdivision raised conservation concerns that this small population was fragmented, furthering increasing extinction risk (Center for Biological Diversity 2008). This finding sparked debate over how to manage this population to prevent further subdivision and increase population connectivity in the future.

My dissertation research uses a combination of genetic and occupancy data collected through a long-term population monitoring program for fisher in the southern Sierra Nevada (Zielinski *et al.* 2013) to better understand how the connectivity and abundance of this population has changed through time. The objectives of my research were to define the timing and cause of isolation of this population, determine the degree of genetic subdivision with the population, identify the landscape features that impede connectivity, and evaluate the ability of the fisher monitoring program to detect population declines.

Research objectives and findings

Historical decline and isolation of fisher in California

In Chapter 2, I use both historical and contemporary genetic samples to determine the timing of the decline and isolation of fisher in the southern Sierra Nevada. The two extant populations of

fisher in California are geographically isolated, separated by more than four times the maximum recorded dispersal distance for fisher (York 1996; Zielinski *et al.* 2005). Their isolation is hypothesized to have resulted from a decline in abundance and distribution associated with European settlement in the 1800s (Zielinski *et al.* 2005). However, there is little evidence to establish that fisher occupied the area between the two extant populations at that time. Determining whether this isolation occurred in recent history or if the population has been persisting in long-term isolation, is an important distinction needed to inform future conservation decisions. I analyzed 275 contemporary and 21 historical fisher samples (1880–1920) at 10 microsatellite loci to evaluate the demographic history of fisher in California. I addressed the following research question:

- Did the isolation of the two California fisher populations occur before or after the European settlement of California?

I find that fisher populations in California experienced a 90% decline in effective population size over 1000 years ago, indicating that the southern Sierra Nevada fisher population became isolated pre-European settlement. I also found a recent bottleneck signal in the northern half of the southern Sierra Nevada fisher population, indicating that a portions of this population experienced another more recent bottleneck post-European settlement, and that the southern tip of the Sierra Nevada may have acted as a refugium for fisher during the anthropogenic changes of the late 19th and early 20th centuries.

This Chapter has been published in *PLOS One* with the title "Historical and contemporary DNA indicate fisher decline and isolation occurred prior to the European settlement of California", co-authored with Fred Allendorf, Mike Schwartz, Kristine Pilgrim, (who provided expertise in the laboratory analysis of historical DNA samples), and Richard Truex (who contributed field samples and expertise on fisher ecology in the southern Sierra Nevada).

Genetic structure of fisher in the southern Sierra Nevada

The objective of Chapter 3 was to determine the population genetic structure of fisher in the southern Sierra Nevada and investigate the influence that sampling can have on the detection of genetic subdivision. A previous genetic analysis found high amounts of subdivision ($F_{ST} = 0.51$) between two sampling areas north and south of the Kings River Canyon (Wisely *et al.* 2004) and inferred that the Kings River could have been a barrier to gene flow between these two sampling areas. However, this study was based on a small number of samples from two limited geographic areas within this population. In this study, I obtained a larger and much more continuously distributed set of genetic samples from the southern Sierra Nevada fisher population. Using 127 individuals genotyped at 10 microsatellite loci I addressed the following research questions:

- What is the genetic structure of fisher in the southern Sierra Nevada fisher population?

- How does sampling scheme (clustered versus continuous) effect the detection of genetic subdivision?

Specifically, I show that population subdivision is much lower than previously thought and that this population is not fragmented into multiple isolated genetic subpopulations. I found three primary genetic subpopulations with moderate divergence between them (F_{ST} = 0.05 - 0.13). These clusters appear to be associated with areas around the Kings River and Mountain Home State Demonstration Forest. I also detected additional fine scale subdivision north of the Kings River that may be evidence of founder effects from a recent population expansion. I showed that the difference in results between this study and previous work are attributable primarily to a difference between clustered and continuous sampling. I found that in a population characterized by isolation by distance clustered sampling can inflate estimates of population structure by oversampling related individuals.

This Chapter is currently submitted and in review with *Conservation Genetics* under the title "Sampling affects the detection of genetic subdivision and conservation implications for fisher in the Sierra Nevada" coauthored by Mike Schwartz, Fred Allendorf, Samantha Wisely, and Richard Truex.

Determining landscape features influencing gene flow

In Chapter 4, I determine what landscape features are creating the genetic subdivision I observed in Chapter 3 using a landscape resistance modeling approach. Genetic connectivity results from the successful dispersal and reproduction of individuals across a landscape. Consequently, landscape features that influence dispersal will also influence gene flow. Sex-biased dispersal, in which one sex is philopatric and the other is more likely to disperse, is a well-documented characteristic of many wildlife populations (Pusey 1987). As male biased dispersal has been documented in other fisher populations (Kelly 1977; Powell 1993; Aubry *et al.* 2005), I hypothesized that the landscape may affect gene flow differently for males than females. The influence of landscape features on dispersal can also vary spatially due to the differential availability of a feature on the landscape (Short Bull *et al.* 2011), or biotic factors such as variation in dispersal pressure (Matthysen 2005) or interspecific interactions (Rundle & Nosil 2005). Therefore, I also tested the hypothesis that the landscape features influencing fisher gene flow will vary by subpopulation. I used genotypic data from 10 microsatellite loci for 72 males and 48 females to address the following research questions:

- Is there genetic evidence of sex-biased dispersal in this fisher population?
- What landscape features influence gene flow?
- Do the landscape features influencing gene flow vary by sex?
- Do the landscape features influencing gene flow vary spatially across the study area?

I found genetic evidence of sex-biased dispersal, with males dispersing between populations in greater proportion than females, and that sex-biased dispersal effects the identification of landscape features influencing gene flow. Specifically, I show that the landscape influences gene flow differently for males and females and that not accounting for this difference when conducting resistance modeling can produce misleading results. I also found landscape features influencing gene flow vary by different geographic regions. Moreover, the landscape variables detected as important when analyzing the entire study area differed from those detected when geographic regions are analyzed separately. After accounting for sex-biased dispersal and landscape heterogeneity in I found much greater gene flow in males compared to females. While female gene flow is impeded by large water bodies and major roads, male gene flow is not affected by these features. Additionally I show that for females gene flow is characterized by dispersal among high quality habitat in the core elevation range for fisher habitat in the Sierra while males disperse more broadly and therefore, strongly associate with a more widespread landcover type.

Factors affecting statistical power to detect trend in occupancy

The objective of Chapter 5 was to investigate the power of long term population monitoring to determine population trend in occupancy. Due to the difficulty of estimating trend using abundance, trend monitoring often relies on estimating trend using occupancy (the proportion of an area occupied by a species) as a surrogate for abundance. The underlying assumption in occupancy monitoring is that a change in the occupancy is indicative of a change in population size but the nature of this relationship will vary depending on the specific characteristics of the species or population (MacKenzie & Nichols 2004). *A priori* power analyses provide a way to assess the statistical power of different sampling strategies to detect population trend. In such situations careful consideration of the factors influencing power is needed in order to best design a sampling strategy to maximize power while minimizing the chance of falsely identifying a trend. Using spatially explicit power analyses I simulated a declining population of fishers in the Sierra Nevada and then recreated the sampling regime of the Sierra Nevada carnivore monitoring program to address the following specific research questions:

- What is the relationship between abundance and occupancy for a declining population?
- How does varying statistical certainty, effective sampling area, and non-continuous sampling effect the power to detect a population trend?
- What is the statistical power to detect a trend in occupancy for the Sierra Nevada fisher carnivore monitoring program?

I found that large declines in abundance (43%) result in relatively small declines in occupancy (23%) but that the rate at which occupancy declined compared to abundance increased over time. I also demonstrate how increasing the effective sampling area, implementing biennial instead of annual sampling, and increasing the type I error rate all increase statistical power to detect trend. Using this simulation approach and mimicking the

sampling of the Sierra Nevada fisher monitoring program, as implemented from 2002-2009 with an average sample size of 140 units/year, I found a 64% power to detect a ~20% decline in occupancy with 20% type I error rate

Synthesis and significance

There is substantial concern that the southern Sierra Nevada fisher population is at risk of extinction stemming from its small population size, isolation, and low genetic diversity. Discussions of how to manage this population to support long-term persistence have included the potential need for translocations or reintroductions into unoccupied areas to expand population size and re-establish genetic and demographic connectivity with other fisher populations (Powell & Zielinski 2005; Sierra Pacific Industries & United States Fish and Wildlife Service 2007). My research shows that this population has been persisting in long-term isolation indicating the potential for significant local adaptations. Consequently, creating genetic connectivity with other fisher populations could actually trigger a reduction in fitness due to outbreeding depression (Edmands 2007; Frankham *et al.* 2011) and that this concern must be balanced along with the risks associated with low genetic diversity and inbreeding depression in management decisions for this population.

Despite the isolation and low genetic diversity of this population, within the southern Sierra Nevada fisher population my research finds that there is a moderate amount of gene flow and that landscape features are not acting as a barrier to genetic connectivity. However, my results also suggest that this population exhibits sex-biased dispersal with males dispersing long distances and females remaining philopatric such that the observed genetic connectivity may largely be the result of male gene flow. Both male and female gene flow is associated with forested habitat with high canopy cover, but female gene flow appears to be limited by major water bodies and roads. This result has important management implications as it indicates that northward population expansion may be limited by the ability of females to disperse across the Merced River canyon and the heavily traveled road system of Yosemite National Park. These results also have broad implications for the field of landscape genetics as I show that failure to account for the presence of sex-biased dispersal can confound the assessment of the landscape features that influence gene flow.

Determining the status and trend of a population is one of the most fundamental tasks in conservation and management and often one of the most difficult to accomplish. For occupancy based population monitoring my research suggests because of the non-linear relationship between abundance and occupancy, this method may only have the power to detect trend for large magnitude declines. This is especially applicable for species such as fisher that occur at low densities across large landscapes and are difficult to sample in large numbers. However, because the rate at which occupancy declines increases over time, the longer a population is

monitored the greater the power it has to detect smaller declines. This emphasizes the importance of long-term population monitoring for conservation and management. Overall the research in my dissertation provides a better understanding of the historical and contemporary connectivity of this population and our ability to monitor population trends over time that will contribute to the conservation of fisher populations in the future.

Dissertation Format

The following chapters are formatted for publication in specific peer-reviewed scientific journals. I use the collective "we" throughout the dissertation to reflect that each of these chapters includes important contributions from many collaborators.

CHAPTER 2: Historical and contemporary DNA indicate fisher decline and isolation occurred prior to the European settlement of California

Abstract

Establishing if species contractions were the result of natural phenomena or human induced landscape changes is essential for managing natural populations. Fishers (*Martes pennanti*) in California occur in two geographically and genetically isolated populations in the northwestern mountains and southern Sierra Nevada. Their isolation is hypothesized to have resulted from a decline in abundance and distribution associated with European settlement in the 1800s. However, there is little evidence to establish that fisher occupied the area between the two extant populations at that time. We analyzed 10 microsatellite loci from 275 contemporary and 21 historical fisher samples (1880-1920) to evaluate the demographic history of fisher in California. We did not find any evidence of a recent (post-European) bottleneck in the northwestern population. In the southern Sierra Nevada, genetic subdivision within the population strongly influenced bottleneck tests. After accounting for genetic subdivision, we found a bottleneck signal only in the northern and central portions of the southern Sierra Nevada, indicating that the southernmost tip of these mountains may have acted as a refugium for fisher during the anthropogenic changes of the late 19th and early 20th centuries. Using a coalescent-based Bayesian analysis, we detected a 90% decline in effective population size and dated the time of decline to over a thousand years ago. We hypothesize that fisher distribution in California contracted to the two current population areas pre-European settlement, and that portions of the southern Sierra Nevada subsequently experienced another more recent bottleneck post-European settlement.[1]

Introduction

Over the past 100 years there has been a marked reduction in many species geographic ranges. For rare or hard to observe species, it is often unclear if their absence is a response to a changing landscape, or if they have been absent from an area for an extended period of time. If they were considered present early during the last epoch, but are now unable to be detected, this is seen as a natural range contraction (Lyons 2003). On the other hand, if they were considered present until the last century, but are now unable to be detected, this is often viewed as caused by human induced disturbances. Establishing if contractions of species were the result of natural causes or human-induced landscape changes is essential for managing natural populations. Mistakes associated with misidentifying the geographic range of a species and misattributing declines in geographic range can have large effects on the allocation of scarce conservation resources.

[1] A taxanomic name change for fisher from *Martes pennanti* to *Pekania pennanti* (Kopefli et al. 2008, Sato et al. 2012) occurred after the publication of this manuscript in the journal *PLoS One.* For this Chapter we have retained the original text as published including the use of *Martes pennanti.*

Traditionally, the historical distribution of a species has been based on accounts of explorers, naturalists, and indigenous peoples that are verified by specimens preserved in museum collections. Recently, technological and laboratory advances in molecular genetics have created the ability to extract DNA from historical specimens and examine the population genetic signals obtained, providing a new tool by which we can test ideas proposed by these early naturalists (Schwartz 2007; Wandeler *et al.* 2007). Historical and contemporary genetic information can provide insight into the nature of population expansions or declines (Goossens *et al.* 2006; Okello *et al.* 2008), the loss of genetic diversity (Smulders *et al.* 2003; Johnson *et al.* 2009), temporal changes in population connectivity (Martinez-Cruz *et al.* 2007), or the historical range of a species (Ross *et al.* 2006; Schwartz *et al.* 2007).

Prior to European settlement, fishers (*Martes pennanti*) were distributed widely in both Canada and the northern U.S. forests (Graham & Graham 1994). In the late 1800's and early 1900's, fisher populations dramatically declined due to a combination of fur trapping, logging, and predator control and by the early 1900's were extirpated from large portions of their historic range (Powell 1993). Reintroductions and expansions from refugia populations have been successful in reestablishing fisher populations in the eastern and Rocky Mountain states (Williams *et al.* 2000; Vinkey *et al.* 2006; Carr *et al.* 2007; Carr *et al.* 2007; Hapeman *et al.* 2011). However, West Coast populations have not experienced the same degree of recovery. There are 5 geographically disjunct fisher populations present on the West Coast: two native populations in California (Zielinski *et al.* 1995; Aubry & Lewis 2003), a reintroduced population established in the 1950's in Oregon, and two recently reintroduced populations (one on the Olympic Peninsula in Washington State and one in California (Lewis & Hayes 2004; Callas & Figura 2008)).

The two native fisher populations in California are geographically and genetically isolated (Zielinski *et al.* 1995; Wisely *et al.* 2004; Knauss *et al.* 2011). Conservation concerns are particularly acute for fisher in the southern Sierra Nevada Mountains because its population size is estimated at less than 300 adults (Spencer *et al.* 2011). The majority of information about the history of fisher in California comes from the work of the naturalist Joseph Grinnell. Grinnell *et al.* (Grinnell *et al.* 1937) used information from extensive surveys, collecting expeditions, trapping records, and local knowledge from approximately 1910-1930 to create distribution maps for 21 species of carnivores. Grinnell's range maps show the historical fisher range as continuous from the northwestern Klamath and Siskiyou Mountains to the southern tip of the Sierra Nevada (Fig. 2-1).

Fisher populations in California are thought to have declined precipitously in both abundance and distribution over the last 150 years due to habitat alteration and fur trapping associated with the European settlement of California beginning with the gold rush in 1848 (Zielinski *et al.* 2005). Currently, the two areas that maintain native populations of fisher in

California are separated by a 420 km gap, which is more than four times the maximum dispersal distance of fisher (Zielinski *et al.* 1995; Zielinski *et al.* 2005). The reason for this gap is not well understood. The majority of habitat in this area is contiguously forested and appears, at least superficially, to be suitable for fisher occupancy. Grinnell's range map shows only a few records of fisher in the central Sierra Nevada and none in the northern Sierra Nevada (Fig. 1), but despite these facts this gap is considered part of the historical range of the species (Grinnell *et al.* 1937).

The accepted hypothesis for the lack of records in the gap area is that the northern and central Sierra Nevada had experienced a greater degree of anthropogenic change at the time of the Grinnell surveys than the southern Sierra Nevada and that the species was already extirpated from the gap by the early 1900's (Zielinski *et al.* 2005). The central and northern Sierra was the main area of human development as a result of the gold rush. Yet, in a study of the history of forest conditions in the Sierra Nevada, McKelvey and Johnston (McKelvey & Johnston 1992) found that due to transportation limitations, logging at the turn of the century was relatively limited in the central and northern Sierra. At this time even the most heavily affected National Forest in this area still had 50% virgin forest and therefore, likely retained areas of large trees that are associated with fisher habitat in California (Zielinski *et al.* 2004; Zielinski *et al.* 2004; Purcell *et al.* 2009). Based on such information, it is unclear why fisher would have been completely extirpated from the gap prior to Grinnell's surveys.

An alternative hypothesis is that this distributional gap may not be the result of recent human influences but rather is a historical discontinuity in fisher distribution that existed prior to the European settlement of California. Fishers are thought to have colonized the West Coast of the United States in a relatively recent range expansion from British Columbia southward in a series of stepwise founder events during the mid to late Holocene (Graham & Graham 1994; Drew *et al.* 2003; Wisely *et al.* 2004). Evidence of an early peninsular expansion is found in the gradient of genetic diversity decreasing from north to south down the West Coast (Wisely *et al.* 2004), and the existence of a shared haplotype between British Columbia and a historical sample from northwestern California (Drew *et al.* 2003). However, evidence indicates there has been little gene flow between the two regions in the time since colonization with high genetic divergence in nuclear DNA ($F_{ST} = 0.48\text{-}0.60$) and the absence of a shared mtDNA haplotype between northwestern California and the southern Sierra Nevada (Wisely *et al.* 2004; Knauss *et al.* 2011).

There are important conservation concerns regarding the southern Sierra Nevada fisher population's risk of extinction stemming from its small population size, isolation, and low genetic diversity. Determining whether the isolation of fisher in the southern Sierra Nevada has occurred recently (within the last 150 years), or if the population has been persisting in long-term isolation, are important alternative hypotheses that need to be distinguished to inform future conservation decisions. Discussions of how to manage this population to support long-term

persistence have included the potential need for translocations to augment populations or reintroductions into the current gap region to re-establish connectivity (Powell & Zielinski 2005; Sierra Pacific Industries & United States Fish and Wildlife Service 2007). If population decline and isolation occurred recently then potential risk from inbreeding depression due to small population size may be an important consideration for the southern Sierra Nevada and aggressive measures to restore genetic connectivity may in fact be prudent. Conversely, detection of a more ancient timeline for isolation would indicate the potential for significant local adaptations within the population and that creating genetic connectivity with northwestern California fishers could actually trigger a reduction in fitness due to outbreeding depression (Edmands 2007; Frankham *et al.* 2011).

Recent research has attempted to address the historical continuity of fisher populations in California using mtDNA. Knaus *et al.* (Knauss *et al.* 2011) sequenced the entire mtDNA genome for 40 fisher samples and found the southern Sierra Nevada to be fixed for a single haplotype that is different from the closest haplotype in northwestern California by 9 base-pair substitutions. The absence of a shared mtDNA haplotype between northwestern California and the southern Sierra Nevada and the amount of genetic differentiation between haplotypes indicates long term isolation. Using a molecular clock approach, they estimated the divergence between these two populations occurred thousands of years ago (Knauss *et al.* 2011).

While the results of Knaus *et al.* (Knauss *et al.* 2011) are striking, mtDNA is maternally transmitted and consequently only provides insight into female mediated gene flow. This may be especially problematic for species such as fisher that exhibit female philopatry where most of the large movements are made by males (Aubry *et al.* 2004). This would result in primarily male mediated gene flow across long distances. As nuclear DNA is bipaternally inherited, it may show different genetic signals from mtDNA that reflect the influence of males on connectivity. Numerous studies have shown discord between estimates of divergence from mtDNA versus nuclear DNA and emphasized the importance of analyzing both mtDNA and nuclear DNA prior to making conservation decisions (Waits *et al.* 2000; Pardini *et al.* 2001; Yang & Kenagy 2009).

Our objective is to use nuclear DNA to distinguish between the alternate hypotheses that the geographic isolation of the two California fisher populations occurred before or after the European settlement of California. We also wish to more precisely date this divergence. The hypothesis that fisher decline and isolation in California occurred prior to 1850 would be supported by lack of evidence of a recent bottleneck and contraction in population size greater than 160 years ago. Conversely, if the hypothesis that isolation occurred after 1850 is correct, we would expect to see evidence of a recent population bottleneck and a contraction in population size within the last ~160 years. Evidence of post-European isolation would be at odds with mtDNA analyses (Knauss *et al.* 2011) and indicate male mediated gene flow between California fisher populations. In a broader sense, this research is also aimed at showing the

importance of understanding historical biogeographic patterns to better understand and manage contemporary patterns of species on the landscape.

Materials and Methods

Ethics Statement

All necessary permits were obtained for the described field studies. These included a Scientific Research and Collecting Permit from the U.S. Department of the Interior, National Park Service (SEKI-2008-SCI-0014).

Samples

We obtained both historical (H) and contemporary (C) genetic samples from the extant range of fisher in California which includes one area in northwestern California (NW) and a second area in the southern Sierra Nevada (SSN) (Fig. 2-2). The NW and SSN populations were defined *a priori* based on previous research that indicated that these populations are geographically isolated due to an unoccupied 420 km gap between them (Zielinski *et al.* 1995; Zielinski *et al.* 2005), as well as genetically isolated (Wisely *et al.* 2004; Knauss *et al.* 2011). We genotyped 127 individuals from hair samples collected in the SSN_C through the U.S. Forest Service Sierra Nevada Carnivore Monitoring Program (Zielinski *et al.* Accepted). In the NW_C we obtained genotypes from 148 individuals based on hair, scat, and tissue samples collected in collaboration with a number of existing research projects in the region. Genetic samples from both regions were collected from 2006-2009. Historical samples were located by searching databases of museum collections. We found 41 fisher specimens from 1884-1920 in the collections of the Smithsonian National Museum of Natural History and the Museum of Vertebrate Zoology at the University of California, Berkeley. We collected maxilloturbinal bones from inside the nasal cavity to maximize the probability of obtaining high quality DNA while minimizing damage to specimens (Fleischer *et al.* 2000; Wisely *et al.* 2004). We also collected tissue from pelts, bone fragments, or muscle when available. In total, 17 historical specimens were obtained from the NW_H and 24 from the SSN_H. We did not find any historical fisher specimens from the current gap in fisher distribution.

Laboratory Analysis

We extracted DNA from museum specimens in a separate laboratory used exclusively for the extraction and processing of genetic material from museum specimens following recommended ancient DNA protocols (Fleischer *et al.* 2000; Gilbert *et al.* 2005). We analyzed the samples at 10 microsatellite loci. *MP0059*, *MP0144*, *MP0175*, *MP0197*, *MP0200,* and *MP0247* were developed from tissue samples from the SSN (Jordan *et al.* 2007). Loci *MA1* (Davis & Strobeck 1998), *GGU101, GGU216* (Duffy *et al.* 1998), and *LUT733* (Dallas & Piertney 1998), were developed in other mustelid species [marten (*Martes americana*), wolverine (*Gulo gulo*), and otter (*Lutra lutra*), respectively].

The quality and quantity of DNA obtained from historical and non-invasive samples can vary considerably because of age and different methods of preservation and storage. The potential for degraded or low quantity DNA increases the likelihood of genotyping errors such as allelic dropout or false alleles (Taberlet & Luikart 1999). To address this potential for error, we ran samples a minimum of three times per locus and accepted genetic data only if the samples produced consistent genotype scores (Eggert *et al.* 2003; McKelvey & Schwartz 2004). If the genotype differed in one or more of these amplifications, we conducted an additional round of 3 amplifications. If multiple inconsistencies were found in the genotype at a locus we removed that sample from the analysis. We also checked for genotyping errors using the software DROPOUT (McKelvey & Schwartz 2005).

Statistical analyses

We tested microsatellite genotypes for departures from Hardy-Weinberg proportions at each locus and gametic disequilibrium for each pair of loci using Fisher's exact test in Genepop 4.0 (Raymond & Rousset 1995; Rousset 2008). We also used Genepop 4.0 to calculate expected heterozygosity (H_E), proportional excess of homozygotes (F_{IS}), F_{ST} (Weir & Cockerham 1984), R_{ST} (Slatkin 1995), and conduct tests for genetic differentiation between sample groups. The amount of genetic diversity present in the sample groups was compared using paired t-tests of arcsine-transformed H_E, and A_R (Archie 1985). We used sequential Bonferroni corrections to correct for multiple comparisons when assessing statistical significance (Rice 1989).

Detecting bottlenecks

We used three methods to determine whether fisher in California had experienced a recent reduction in population size. We first tested for heterozygosity excess which is characteristic of bottlenecked populations using BOTTLENECK 1.2.02 (Piry *et al.* 1999). This heterozygosity excess exists because rare alleles are lost more rapidly during a bottleneck but have little impact on heterozygosity (Cornuet & Luikart 1996). Heterozygosity excess is transient and will only persist for $0.2 – 4N_e$ generations after the bottleneck. The average expected heterozygosity at mutation-drift equilibrium was calculated using 5000 replications assuming a two-phase mutational model. We conducted analyses with both 5% and 20% of mutations set as multistep mutations in the two-phase model with a variance of 12 to encompass the range of multistep mutations observed in natural populations (Di Rienzo *et al.* 1994). The observed heterozygosity was then tested against the equilibrium expected heterozygosity using the Wilcoxon signed-rank test. We also conducted the test excluding all loci that were out of Hardy-Weinberg, as such loci can create bias, but doing so did not significantly change the results.

Second, we also used BOTTLENECK to test for a shift in the mode of the distribution of allele frequencies. This mode shift distortion is transient and can only be detected for a few dozen generations. Luikart *et al.* (Luikart *et al.* 1998) found using simulations that the graphical mode shift method is likely ($P > .80$) to detect a bottleneck of up to 20 breeding individuals using

8-10 microsatellite loci. The mode shift test could not be applied to the historical samples because at least 30 individuals are needed to avoid high type 1 error rates.

The third method used detects reductions in effective population size (N_e) using the *M*-Ratio which is defined as $M = k/r$ where k is the total number of alleles and r is the range in allele size (Garza & Williamson 2001). Because a bottleneck causes a greater reduction in the number of alleles than in the range of allele sizes, *M* is smaller in reduced populations. Garza and Williamson (Garza & Williamson 2001) found that a reduction in population size can be detected using *M* for 125 generations if the population rebounded quickly in size or 500 generations if the population remained reduced. We used the software *M*_P_Val to calculate *M* and the software *M*_Critical to determine the cutoff value for statistical significance (Garza & Williamson 2001). We set model parameters at 90% single-step mutations and 10% multi-step mutations (p_s) and the average size of multistep mutation (Δg) of 3.5 with the mutation rate μ held constant at 5×10^{-4}. In this model $\theta = 4N_e\mu$ so if μ is held constant different values of θ are representative of different starting (pre-decline) N_e. As the equilibrium N_e for fisher in California is not known, we calculated *M* and *M*-Critical values for four different values of θ (1, 2, 5, and 10) which represent a wide range of pre-decline N_e (500, 1000, 2500, and 5000 respectively).

The presence of unaccounted for genetic subdivision has the potential to bias bottleneck tests (Broquet *et al.* 2010). While genetic subdivision has not been previously detected in the NW_C population, past research has shown significant subdivision in the SSN_C (Wisely *et al.* 2004). To assess the influence of this subdivision, we divided the SSN_C into three genetic groups and assessed the influence of this on the bottleneck tests. The subdivisions between demes in the SSN_C roughly correspond to the areas north of the Kings River (North), between the Kings River and Middle Fork of the North Fork of the Tule River (Central), south of the Middle Fork of the North Fork Tule River (South) (Fig. 2-3). Previous research on fisher populations in southern Ontario has found rivers to be a major barrier to genetic connectivity (Garroway *et al.* 2011; Hapeman *et al.* 2011). These subdivision boundaries are also supported by data from a recent population genetic analysis of the SSN_C showing moderate subdivision (F_{ST} 0.05-0.13) between these areas (J.M. Tucker unpublished data).

Demographic history models

We employed a coalescent-based Bayesian analysis to assess the most recent major change in N_e and to estimate the date of the change. This model assumes that an ancestral N_e (N_1) changed to the current N_e (N_0), at a time *T* generations ago (Beaumont 1999; Storz & Beaumont 2002). This model uses a stepwise mutation model and assumes a mutation rate scaled in terms of the current populations size such that that $\theta=2N_0\mu$, where μ is the per locus mutation rate. While this method does employ a strict stepwise mutation model, it has been found to be robust to moderate departures created by the presence of multistep mutations (Girod *et al.* 2011). The method then estimates the posterior distributions of N_1, N_0, *T*, and θ that describe the genealogical and

demographic history of the sample, assuming either linear or exponential size change. Prior distributions for N_1, N_0, T, and θ are assumed to be log normal with their means and standard deviations drawn from hyperprior distributions truncated at zero. We conducted the analysis using MSVAR 1.3 (Storz & Beaumont 2002) which uses Markov Chain Monte Carlo (MCMC) simulations to estimate the posterior distribution of each parameter.

We conducted 6 independent simulations of the model varying the prior and hyperprior distributions with a range of biologically realistic distribution values to examine their effect on the posterior distributions. These variations of the priors had little effect on the posterior distribution of the models so prior distributions for all other analyses were set to the parameters of simulation 1. To check for the convergence of model we conducted five replications of the simulations for each data set. Each simulation was performed for 2×10^9 iterations with parameter values recorded every 1×10^5 iterations resulting in 20,000 records.

We removed the first 10% of data from each chain as burn-in and assessed chain convergence using the Brooks, Gelman, and Rubin Convergence Diagnostic test (Gelman & Rubin 1992; Brooks & Gelman 1998). We conducted convergence diagnostics in R version 2.11.1 (R Development Core Team 2012) using the package BOA version 1.1.7 (Smith 2007). The test statistic is a multivariate potential scale reduction factor (MPSRF) that assesses the convergence of a set of parameters simultaneously. The MPSRF value for all parameters was ~1.0 indicating acceptable chain convergence. We then combined the last 50% of the data from each chain (10,000 records/chain, 50,000 total records) and calculated the mode and 90% highest posterior densities (HPD) of the posterior distributions of each parameter using the R-package Locfit 1.5-6 (Loader 2007). We evaluated the strength of evidence for population expansion versus decline by calculating the Bayes factor for each of the models (Jeffreys 1961; Kass & Raftery 1995) as described by Storz and Beaumont (Storz & Beaumont 2002). The Bayes factor indicates the following levels of support for the model; BF<0.33= false detection of contraction/expansion, 0.33-3=no support, 3-10=substantial support, and ≥10=strong support (Jeffreys 1961).

While the generation time (average age of reproduction) for fisher has not been well studied, the average age of first reproduction is estimated at 2-3 years, with high reproductive rates documented in 5-7 year old females [76], and successful reproduction found in females as old as 10 years (C. Thompson personal communication). We used a generation interval of five years. Parameter estimates of T can easily be adjusted for different generation times by multiplying accordingly. We ran the simulations for all data sets using both the exponential and linear models.

Results

We successfully obtained genotypes at a minimum of seven loci for 127 individuals in the SSN_C, 148 individuals in the NW_C, 16 individuals from the SSN_H, and five individuals from the NW_H (Table 2-1). The dates of the historical samples that successfully yielded microsatellite genotypes ranged from 1884-1920, which represents the overall timeframe of available historical samples (Table 2-S1). Nine of the 10 microsatellite loci were polymorphic in all samples. The exception was the *MA1* locus which was monomorphic in the NW_C. Tests for Hardy-Weinberg proportions showed deviation from expected values at *MP200* and *MP59* in the SSN_C. However, these deviations are non-significant after accounting for genetic population structure. We also found *MP200* deviated in the SSN_H to have a homozygote excess compared to expected Hardy-Weinberg proportions. To assess the influence of this locus, we conducted SSN_H analyses both with and without this locus but did not find any notable difference in results.

While we did not find any evidence for departure from Hardy-Weinberg proportions at individual loci, we did find some important patterns over all loci within each sample group. F_{IS} values were small and statistically insignificant in both the NW_H and NW_C samples, but had significant p-values in both the SSN_H and SSN_C. Most notably, the SSN_C showed a large deficit of heterozygotes (F_{IS} = 0.101, p<0.001) (Table 2-1). This is indicative of the potential presence of the Wahlund effect (Wahlund 1928) in the SSN, in which unaccounted for population subdivision in a sample generates a deficit of heterozygotes relative to expected Hardy-Weinberg proportions.

Tests for gametic disequilibrium did not find any strong associations between loci. After correcting for multiple comparisons statistically significant gametic disequilibrium was found between two pairs of loci in the SSN_C (*MP197*/*MP200*, and *MA1*/*MP144)*, one pair in the NW_C (*MP175*/*LUT733),* and none in either historical sample group. No pairs of loci were consistently significant across sample groups indicating that the loci used were assorting independently.

We did not find any difference in the amount of genetic diversity within sample groups with paired t-tests showing no significant differences in H_E, or A_R. However, all metrics of genetic diversity were lowest in the NW_C (Table 2-1). H_E was markedly lower in the NW_C (0.431) compared to all other samples (0.57-0.64). Allelic richness (A_R) was higher in both historical samples (NW_H =3.34, SSN_H =2.81) than in either contemporary sample (NW_C =2.17, SSN_C =2.51). Samples in the NW_C were monomorphic at locus MA1, and have extremely low diversity at the MP200 locus (2 of 3 alleles at 1% frequency). When these two loci were removed from calculations the NW_C H_E increases to 0.54 which is similar to the value for the other sample groups at 8 loci (NW_H = 0.55, SSN_H = 0.60, SSN_C = 0.55) and A_R in the two contemporary populations becomes equal ($SSN_{C(8loci)}$ = $NW_{C(8loci)}$ = 2.46).

We found each group to be significantly genetically different. Tests for genic differentiation between sample groups were significant at P<0.001. F_{ST} and R_{ST} values were moderate between historical sample groups (NW_H/ SSN_H: F_{ST}= 0.10, R_{ST} =0.20) but increased over time with contemporary samples showing increased divergence (SSN_C/NW_C: F_{ST} = 0.37, R_{ST}= 0.58). We also found temporal divergence over time with moderate F_{ST} values between temporally spaced samples in the same geographic location (SSN_H/SSN_C = 0.17, NW_H/NW_C = 0.20) (Table 2-2). R_{ST} values were considerably higher than F_{ST} values indicating that when variation in allele length is accounted for genetic divergence between samples groups is even greater.

Population bottlenecks

We did not find any signal of a recent population bottleneck for either the historical or contemporary NW samples. Both NW samples had non-significant results for the Wilcoxon heterozygosity excess test and the NW_C was also negative for the shifted mode test. Bottleneck tests for the SSN were mixed. For the SSN_C the heterozygosity excess test was statistically significant regardless of the proportion of multistep mutations in the two-phase model (5%: p=0.04, 20%: p<0.001), but showed no evidence of a shifted mode. The SSN_H was significant at α=0.05 for heterozygosity excess but only when using 20% multistep mutations (5%: p=0.10, 20%: p=0.05). We found no evidence of a population bottleneck for any sample group using the *M*-Ratio method (Table 2-3).

The mixed results in the SSN_C were clarified after accounting for genetic population subdivision. Both the North and Central SSN_C samples showed strong evidence of a recent bottleneck with significant heterozygosity excess tests (p<0.001) and shifted modes. The South SSN_C sample showed no evidence of a recent bottleneck in either the heterozygosity excess or shifted mode tests. After accounting for populations subdivision there was still no evidence of a bottleneck in the SSN_C using the *M*-Ratio method (Table 2-3).

Demographic history

We were unable to obtain consistent results for the demographic change analysis in the NW_H due to small sample size (n=5) and therefore, did not include these results in our analyses. However, results from the other three sample groups consistently indicate that there was a large population decline with current N_e estimates over 90% lower than the estimates of the ancestral N_e. These results were consistent across a variety of prior distributions and both demographic models (exponential and linear). Bayes factor values were >10 for all models indicating strong evidence for a population decline (Table 2-4).

The ratio of the posterior distributions of current and ancestral population sizes ($r=N_0/N_1$) indicates the direction of demographic change where r=1 signifies population stability, r<1 population decline, and r>1 population expansion. Combining all simulations for all data sets for

the exponential model we found the 90% highest posterior density (HPD) of the ratio r to be 0.011 – 0.095 with a mode of 0.081, and for the linear model an HPD of 0.010 – 0.066 with a mode of 0.062. These r values indicate that the current N_e is estimated to be less than 10% of the ancestral N_e and show an unambiguous signal of population decline for fisher in California (Fig. 2-4A).

The modes of the 90% HPD of the posterior distributions for ancestral effective population size (N_1) for the exponential model were SSN_H =1862, SSN_C = 1613, and NW_C =1698 compared to modal values for current effective population sizes (N_0) of 154, 167, and 129 respectively (Table 2-4, Fig. 2-4 B-D). Estimates for N_0 and N_1 were similar but slightly lower for the linear model. Estimates of the time of population contraction varied between populations, but all showed support for population decline occurring well prior to the European settlement of California (T- SSN_C = 1693 years before present [YBP], T-NW_C = 2884 YBP, T-SSN_H = 442 YBP). We adjusted the time estimates for the SSN_H data to reflect the increased age of samples by adding the average age of the sample (95 years) to the estimate. Estimates for the timing of the decline were longer for the linear model than for the exponential model for all sample groups (Table2-4, Fig. 2-5). We put more emphasis on the results of the exponential model because it is likely more realistic when modeling population dynamics (Beaumont 1999).

Population subdivision can also bias demographic history models by creating a spurious signal of population decline. The potential bias is greatest for highly subdivided populations (high F_{ST}), highly variable markers, and species with large N_e (Chikhi *et al.* 2010). The recommended ad hoc method to counteract any potential bias created by population subdivision is to sample equally across demes (Chikhi *et al.* 2010). We followed this ad hoc approach by conducting the MSVAR analysis in the SSN_C with numerous samples from all three of the identified demes such that each of the North, Central, and South groups were well represented in our sample. Considering the characteristics of the data used in this analysis (moderate F_{ST} values, low variability markers, and small population size) reduce the potential for biased results, combined with our use of the ad hoc method of sampling across demes, we feel our results are robust to the potential bias created by population subdivision.

Discussion

Population contraction and isolation

Our analyses supports the hypothesis that the NW and SSN fisher populations became isolated far before the European settlement of California and that the absence of fisher in the northern Sierra Nevada is likely a long standing gap in this species' historical range. We found a genetic signal for a more than 90% reduction in N_e of fisher and estimated that this decline occurred over a thousand years ago. A decline of this magnitude is consistent with a major range contraction. There is a positive correlation between changes in abundance and distribution, where species' abundance decreases its range also decreases (Fuller *et al.* 1995; Gaston *et al.* 1997; Newton

1997; Rodríguez 2002); species with the strongest declines exhibit the largest range contractions (Fuller *et al.* 1995). While the positive correlation between abundance and range size is not universal (Fuller *et al.* 1995; Gaston & Curnutt 1998), the extreme decline in N_e detected in our analyses makes the idea of concurrent stability in range size unlikely. While the 90% highest posterior density of 3 of the 6 models did not definitively exclude a post-settlement decline (Table 2-4), the vast majority of the mass of the distribution of the time parameter (T) support pre-European settlement, with an average of 90% of the contemporary and 81% of historical MCMC chains indicating a time of contraction prior to 1850.

In addition to an ancient population contraction that isolated the SSN from the NW, our analyses indicate the SSN has also undergone a more recent population bottleneck likely associated with the impact of human development in the late 19th and early 20th century. The presence of a bottleneck signal only in the north and central portions of the SSN_C and not in the south reflects differences in the extent of anthropogenic influence across the Sierra Nevada. The majority of human settlement, and its associated impacts, occurred in the central and northern Sierra Nevada. Settlement in the southern Sierra was minimal in comparison due to the absence of gold deposits and steeper topography that restricted access to forest lands. Our results indicate that the area at southern tip of the Sierra Nevada may have acted as a refuge for fisher during the era of extensive logging and development that began with the gold rush and continued into the first half of the twentieth century (Beesley 1996). This area appears to have maintained a stable population size while fisher in the rest of SSN was in decline.

The window of time that the heterozygosity excess and shifted mode tests can detect a bottleneck is shorter than the timeframe for the *M*-Ratio test. The magnitude of the reduction in the *M*-Ratio from equilibrium values is also highly dependent on the pre-bottleneck population size. Accordingly, simulation studies have shown the *M*-Ratio test performs well if the pre-bottleneck population size was large, the bottleneck was of long duration, or the population had time to recover (Williamson-Natesan 2005). The length of time that the *M*-Ratio is informative can vary considerably (125-500 generations) depending on the bottleneck characteristics in terms of severity, duration, and post-bottleneck recovery. Assuming a generation interval for fisher of 5 years, significantly reduced *M*-Ratios would be indicative of decline that occurred anywhere from 625-2500 years ago. However, in permanently reduced populations the *M*-Ratio will recover over time, whereas allelic diversity does not (Garza & Williamson 2001). Consequently, a population with low allelic diversity but a high *M*-Ratio, such as was observed in this study, is indicative of a population that has been small for a very long time. This conclusion is further supported by the fact that we found all sample groups to have low genetic diversity, and did not find any significant difference in diversity between contemporary and historical samples (collected between 1880 and 1920). This suggests that a population reduction, and its concurrent reduction in genetic diversity, occurred prior to the dates of the historical samples.

Our data suggests continual isolation of the NW and SSN populations during the last century. The increase in F_{ST} from 0.10 in the early 1900s to 0.37 in 2006-2009 shows the genetic isolation of the populations during the intervening years. However, the F_{ST} estimates between historical NW and SSN samples are likely biased considering the number of samples available from each population was small and from a relatively limited geographic subset of each area. Genotypic differentiation was strong across all spatial and temporal samples, and the amount of within population genetic differentiation over time period was similar in both areas (F_{ST}: SSN_H-SSN_C = 0.17, NW_H-NW_C = 0.20) which can be attributed to the effects of genetic drift in small populations over time.

Considerations for bottleneck tests

Recent studies have found that bottleneck detection methods sometimes perform poorly at detecting very recent or weak population declines (Mock *et al.* 2004; Busch *et al.* 2007; Girod *et al.* 2011). This creates a concern that a post-settlement decline would not be detected even if it had occurred. Girod *et al.* (Girod *et al.* 2011) used simulations to evaluate the ability of MSVAR to detect expansion/declines assessing performance using Bayes factors. Their analyses of populations with recent and/or weak declines resulted in very low Bayes factors (≤3) indicating no support for the detection of a decline. Accordingly, if the decline in the California fisher population was very recent we would expect MSVAR to produce a model with little support (low Bayes factors) reflecting the supposed poor ability of the method to detect recent declines. However, our MSVAR analyses produced high Bayes factors (≥10) for all models showing strongly supported signals of decline. Such high Bayes factors are in agreement with the results of the Girod *et al.* (Girod *et al.* 2011) for more ancient times of contraction (≥50 generations). The poor performance of the heterozygosity excess and *M*-Ratio tests detected in the Girod *et al.* (Girod *et al.* 2011) study is likely due to their simulation being conducted under a strict stepwise mutational model which has been identified as an unrealistically conservative model for microsatellite loci that may not have much power to detect bottlenecks that have actually occurred (Luikart & Cornuet 1998). Other studies have shown these two methods to have a much higher power to detect bottlenecks (Luikart & Cornuet 1998; Williamson-Natesan 2005).

An important consideration in the interpretation of bottleneck tests is the potential influence of isolation by distance (IBD) within populations. While the SSN_C has been found to exhibit a significant isolation by distance pattern across the entire population, tests for IBD were non-significant within each of the North, Central, and South subpopulations (J.M. Tucker unpublished data). The clustered distribution of samples in the NW_C and SSN_H and the small sample size of the NW_H prevented us from testing for IBD in these populations. However, IBD has been found to have little effect on the heterozygosity excess method implemented in BOTTLENECK (Leblois *et al.* 2006). IBD does influence the *M*-Ratio such that both equilibrium and post bottleneck values of *M* are depressed compared with a non-IBD population. Thus, IBD can result in M values in non-bottlenecked populations that are lower than the Garza

and Williamson's (Garza & Williamson 2001) recommended *M*-Critical cutoff value of 0.68 providing a false signal of a bottleneck. However, given the consistently high *M* values detected in this study (M = 0.82-0.92, Table 3) we do not feel that IBD biased our *M*-Ratio analyses.

Effective population size estimates

The similarity between the estimates of N_e in the NW_C and SSN_C populations is surprising given that the NW_C is thought to have a larger total population size (*N*) than the SSN_C. There are no published estimates of *N* in the NW_C, but unofficial estimates place it at between 1000-2000 individuals (C. Carroll personal communication cited in (Greenwald *et al.* 2000)) compared to estimates of 160-360 for the SSN_C (Spencer *et al.* 2008). The ratio of N_e/N is not well understood and can vary considerably between populations or species due to factors such as fluctuating population size, variance in reproductive success, unequal sex ratio, or population density (Frankham 1995; Wade 1980; Ardren & Kapuscinski 2003; Hare *et al.* 2011). Predicted values of the N_e/N ratio in the literature vary widely; Nunney (Nunney 1993) estimated that theoretically the N_e/N ratio should be 0.5, Nunney and Elam (Nunney & Elam 1994) found the average ratio across empirical data from 13 species to be 0.73, and Frankham (Frankham 1995) found the mean ratio across 102 species to be 0.11. Consequently, it is difficult to interpret what the estimated values of N_e mean in terms of *N* in relation to each population. Extrapolating the modal values of the exponential model for N_0 across a wide range of possible N_e/N ratio values of 0.05 – 0.5, the total population size for the NW could range from 258-2850 and SSN from 334 – 3380. Both of these population size ranges encompass the current possible estimates of *N* for both areas.

Biogeographical influences

The population contraction detected in this study and in the ancient mitochondrial divergence date reported by Knaus *et al.* (Knauss *et al.* 2011) may reflect a shift in habitat distribution or community composition associated with one of a number of potentially significant climate shifts during the end of the Pleistocene or Holocene epochs. There are many well-known hypotheses about the cause of the mass extinctions and major shifts in species distribution that occurred at the end of the Pleistocene including temperature increases, changes in precipitation, or shifts in the ecological balance due to the arrival of human hunters in North America (Barnosky *et al.* 2004). In more recent climactic history there are two well documented "mega-droughts" that occurred in California that have not been matched in severity or duration since. These droughts were first described by Stine (Stine 1994) and were estimated to have lasted over 200 and 140 years each from 832-1074 and 1122-1299 AD respectively (Cook *et al.* 2010). These droughts fall into a period of warmer temperatures referred to as the Medieval Warm Period (Lamb 1965) or Medieval Climate Anomaly (Stine 1994). While the divergence dates reported by Knaus *et al.* (Knauss *et al.* 2011) would support a late Pleistocene climate shift as a possible cause of the divergence of California fisher populations, the results of this study found dates that support a more recent event, such as the aforementioned mega-droughts as a potential cause of the

population contraction. Neither method allows for precise dating of the demographic shift. Nevertheless, both studies show that the contraction of the fisher populations in California pre-dated the gold rush and was not a direct result of the European settlement of California.

The reason fisher would be absent from the central and northern Sierra is perplexing, considering that there is no obvious geographic feature that marks a significant break in the topography or vegetative composition of the Sierra Nevada. However, a number of other species such as the great gray owl (*Strix nebulosa*), wolverine (*Gulo gulo*), and foxtail pine (*Pinus balfouriana*) have also been found to have long term genetic and geographic isolation in the southern Sierra Nevada (Hull *et al.* 2010; Schwartz *et al.* 2007; Eckert *et al.* 2008) indicating that there are perhaps unique vegetative, climactic, or topographic elements in this region that are absent from the northern Sierra Nevada. A recent climate assessment has shown the southern Sierra Nevada to be somewhat resistant to climate changes observed elsewhere in California due to the extreme elevation of the mountains in this region (Moser *et al.* 2009).

The Sierra Nevada is characterized by a gradual change in its maximum elevation and average slope, such that the elevation of the Sierran crest and average slope is highest in the south. The area of the Sierra Nevada occupied by fisher is at the southernmost extent of its range where the weather is hotter and drier than in other areas. To mitigate the effects of high heat and low humidity, fisher may use cool and damp microhabitats characterized by dense canopies, large diameter trees, steep slopes, and close proximity to water (Zielinski *et al.* 2004). One possible explanation for fisher presence in the southern Sierra is that the steep topography in this portion of the mountain range facilitates the creation and persistence of these essential microhabitat areas.

Relatively high amounts of subdivision have been reported in other parts of the fisher's range. Kyle *et al.* (Kyle *et al.* 2001) found the amount of genetic subdivision observed between fisher populations (global F_{ST}= 0.137) was much higher than for other closely related carnivore species of American marten (F_{ST}=0.0198) or wolverine (F_{ST}=0.0427) (Kyle *et al.* 2000; Kyle *et al.* 2002). A linear regression of genetic versus geographic distance found that fisher have twice the subdivision per unit distance than martens and 5 times more per unit distance than wolverine (Kyle & Strobeck 2001). The high amounts of subdivision observed in fisher may result from being habitat specialists which makes them especially vulnerable to habitat fragmentation (Buskirk & Powell 1994; Weir & Corbould 2010). Strikingly, this study found the structure per unit distance between the SSN_C and NW_C to be to be ~10 times greater (0.961/1000 km) and between then SSN_H and NW_H to be ~4 times greater (0.348/1000 km) than Kyle *et al.* (Kyle *et al.* 2001) found for fisher populations across North America (0.092/1000 km). However, high subdivision is not universal among fisher populations. Populations in southern Ontario, Canada have been found to have weak subdivision and high genetic connectivity attributed to high

amounts of gene flow along expansion fronts in a growing population (Carr *et al.* 2007; Garroway *et al.* 2008).

Conservation Implications

Our results provide a historical perspective for contemporary conservation and management decisions for fisher in California. There are ongoing debates as to whether efforts should be made to restore connectivity between the NW and SSN and thereby increase genetic diversity in the isolated SSN. The results of this study show that both populations have persisted in isolation far prior to the European settlement of California. Therefore, attempting to restore connectivity between them would be inconsistent with the historical record and run the risk of losing local adaptations that evolved in each population (Frankham *et al.* 2011). Given their long term isolation, the NW and SSN fisher populations should be considered independently for management and conservation decisions**.**

In 2004, the west coast population of fisher (southern Oregon, northwestern California, and southern Sierra Nevada of California) was found warranted but precluded for listing as a single distinct population segment (DPS) under the federal Endangered Species Act (U.S. Department of the Interior, Fish and Wildlife Service 2004). Among the criteria for considering a population as a DPS it must be markedly separated from other populations of the same taxon (discrete) and differ from other populations in its ecological setting or genetic characteristics (significant) (United States Fish and Wildlife Service & National Marine Fisheries Service 1996). As both of these criteria can be met by quantitative measures of genetic discontinuity or genetic uniqueness (United States Fish and Wildlife Service & National Marine Fisheries Service 1996), the detection of long term genetic isolation of the southern Sierra Nevada fisher population has important implications for its legal status. The observed genetic differentiation coupled with observed differences in diet, home range size, and habitat associations between the SSN and NW (Zielinski *et al.* 1999; Zielinski *et al.* 2004; Davis *et al.* 2007) speaks to the potential of the SSN population itself as a DPS.

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Table 2-1. Estimates of genetic diversity for the northwest (NW) and southern Sierra Nevada (SSN) at 10 microsatellite loci: sample size (n), expected heterozygosity (H_E), proportional excess of homozygotes (F_{IS}), mean number of alleles (A), and allelic richness (A_R). Allelic richness is based on a minimum size of 4 individuals that represents the number of individuals with genotypes at all 10 loci in the historical NW sample.

	n	H_E	F_{IS}	A	A_R
NW-Historical	5	0.635	0.028	3.60	3.34
NW-Contemporary	148	0.431	0.028	3.75	2.17
SSN-Historical	16	0.590	0.046**	3.60	2.81
SSN-Contemporary	127	0.565	0.101***	3.50	2.51

** P< 0.05; *** P < 0.001

Table 2-2. Pairwise comparisons of genetic differentiation between samples with R_{ST} above the diagonal and F_{ST} below the diagonal. H denotes historical samples and C denotes contemporary samples. All pairwise comparisons shown in the table are significant at P<0.01.

	NW_H	NW_C	SSN_H	SSN_C
NW_H	--	0.321	0.195	0.500
NW_C	0.198	--	0.363	0.581
SSN_H	0.098	0.291	--	0.265
SSN_C	0.208	0.374	0.170	--

Table 2-3. Results of BOTTLENECK tests including the p values for the Wilcoxon heterozygosity (H_E) excess test with two different proportions of multistep mutations in the two phase model (TPM), shifted mode test, and *M*-Ratio value and *M*-Critical values. θ=1 represents an initial (pre-decline) N_e of 500 and θ=10 an N_e of 5000. *M*-Ratio values that fall below the *M*-Critical value are considered statistically significant at α=0.05. Results incorporating population structure in the SSN_C are shown on the last 3 lines where H denotes historical samples and C denotes contemporary samples.

	n	H_E Excess TPM 20%	H_E Excess TPM 5%	Shifted Mode	*M*-Ratio	*M*-Critical θ=1	*M*-Critical θ=10
NW_H	5	0.19	0.22	-	0.92	0.71	0.55
NW_C	148	0.08	0.22	No	0.91	0.77	0.71
SSN_H	16	0.05	0.10	-	0.87	0.78	0.64
SSN_C	127	0.00	0.04	No	0.89	0.78	0.72
SSN_C – North	44	<0.001	<0.001	Yes	0.83	0.78	0.69
SSN_C – Central	32	<0.001	<0.001	Yes	0.82	0.78	0.68
SSN_C – South	51	0.08	0.19	No	0.85	0.78	0.70

Table 2-4. The mode and 90% highest posterior density (in parentheses) of the posterior distributions for the Storz and Beaumont [67] models. The Bayes factor (BF) indicates the strength of evidence for a population decline with values greater than 10 representing very strong support. N_0 and N_1 are the current and ancestral N_e respectively. Time (T) represents the date of the change in population size from N_0 to N_1.

Sample	BF	Scale	N_0	N_1	Time (T)
Historical-SSN	10.9	**Exp**	**154 (1-2160)**	**1862 (454-7952)**	**442 (96-25249)**
	13.5	Linear	102 (1-1993)	1922 (457-7838)	1054 (109-61941)
Contemporary-SSN	36.1	**Exp**	**167 (23-838)**	**1613 (383-7102)**	**1693 (60-23307)**
	65.2	Linear	139 (17-692)	1405 (358-8143)	3134 (160-73610)
Contemporary-NW	41.1	**Exp**	**129 (23-513)**	**1698 (288-12302)**	**2884 (162-37153)**
	45.5	Linear	128 (27-547)	1640 (246-19639)	8549 (373-353012)

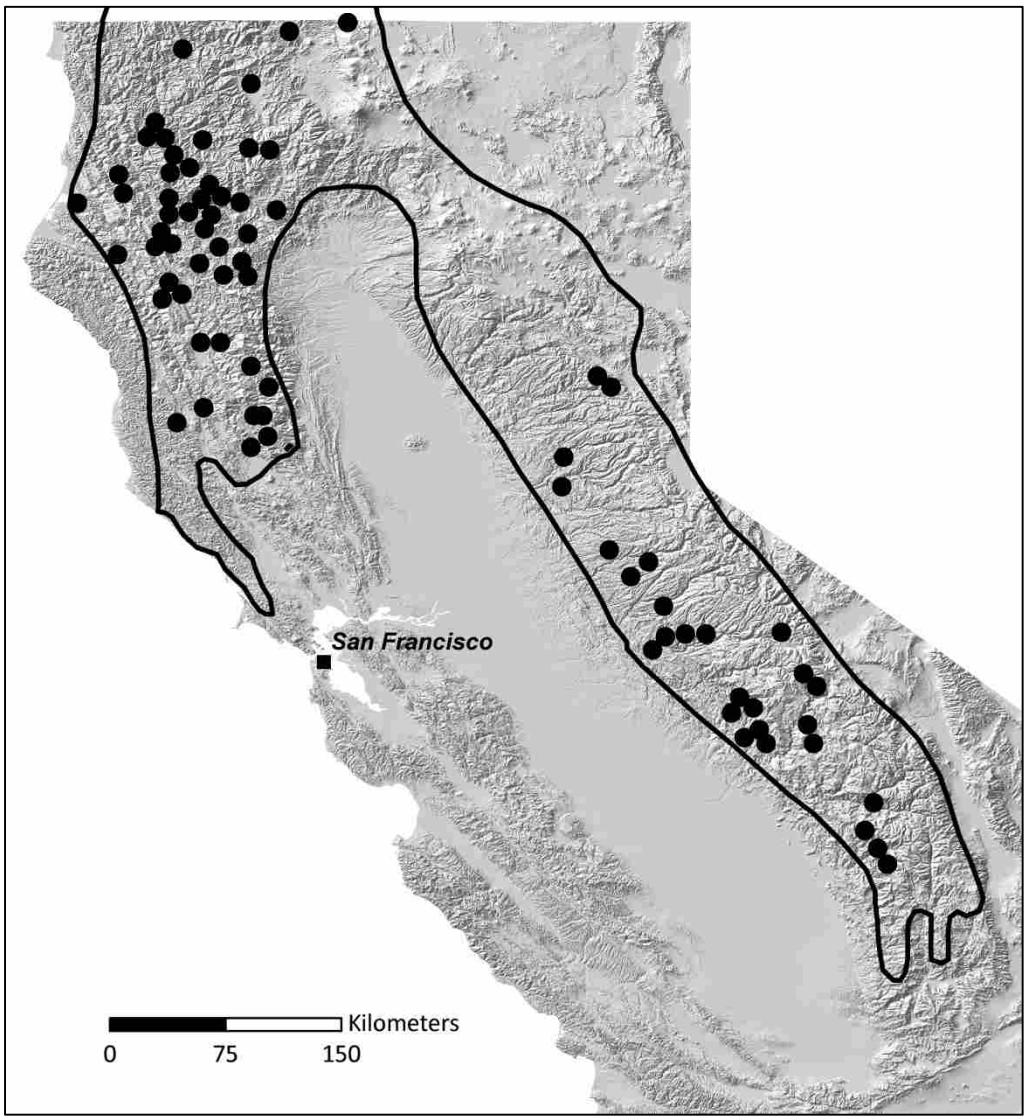

Figure 2-1. Fisher locations used by Grinnell *et al.* (1937) to document the distribution of fisher in California. Locations are based primarily on reports of trappers and collecting expeditions from 1919-1924. Grinnell wrote that "spots [black dots] indicate, almost all of them with certainty, the locality of capture; probably some indicate the residence of post office or trapper". The outlined area is the Grinnell *et al.* (1937) assessment of the range of fisher in California from ~1850-1925.

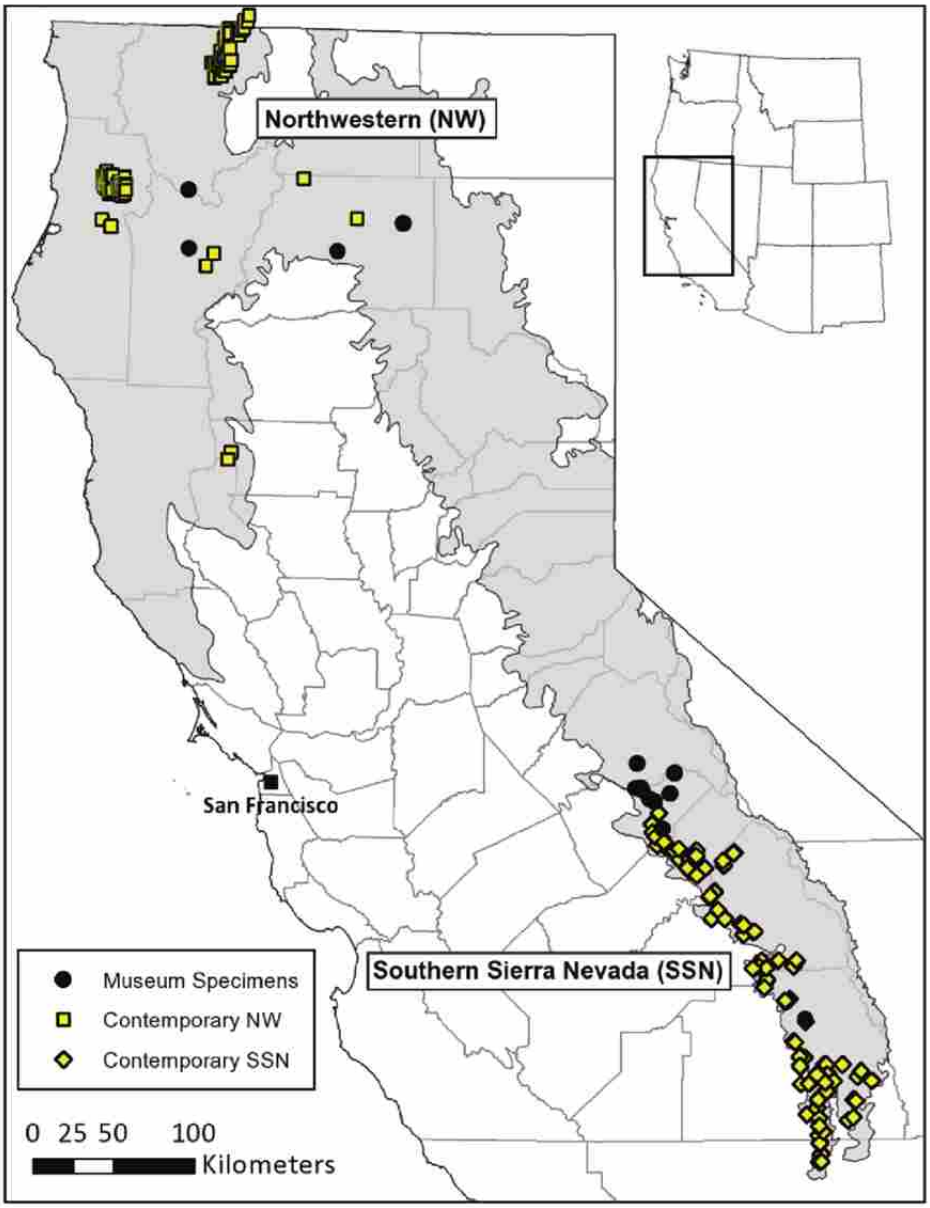

Figure 2-2. Locations of the historical (H) and contemporary (C) genetic samples from the northwestern mountains (NW) and southern Sierra Nevada (SSN) of California. Sample size is as follows: NW_H n=5, SSN_H n=16, NW_C n=148, SSN_C n=127. Grinnell's assumed historical range as adapted by Davis *et al.* [115] is shown in gray.

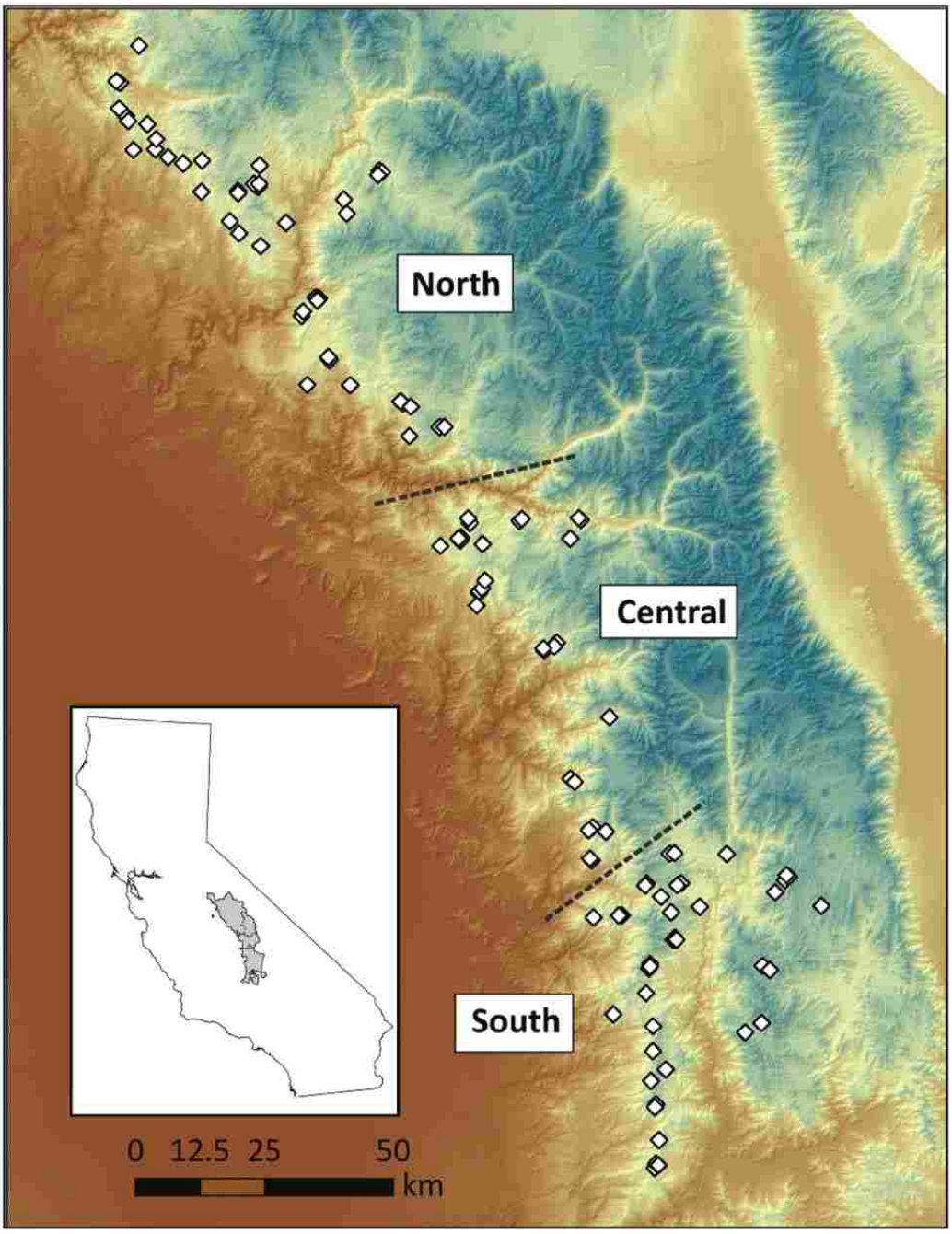

Figure 2-3. Approximate location of population subdivisions used in bottleneck analyses within the contemporary southern Sierra Nevada.

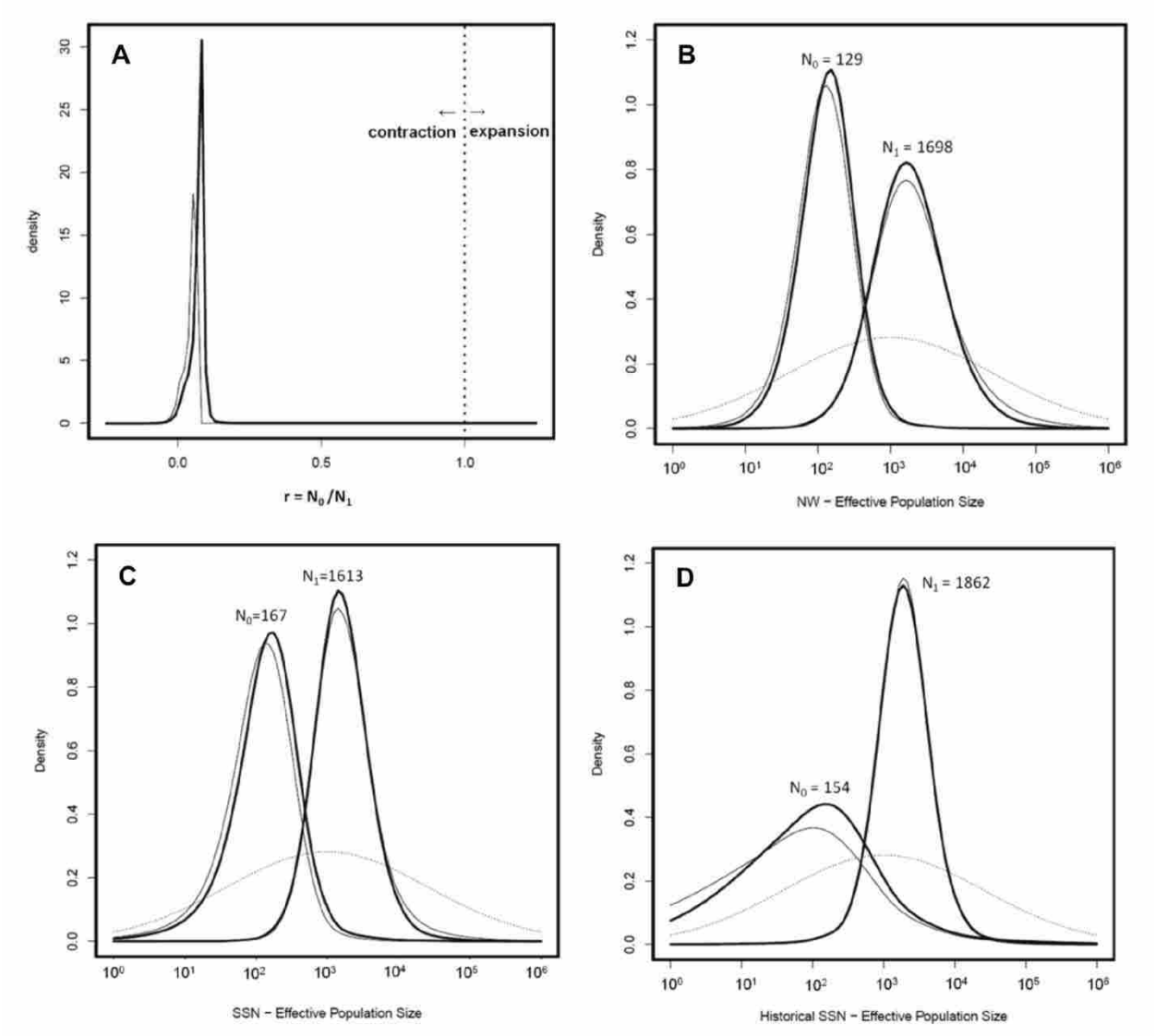

Figure 2-4. A) ratio of current and ancestral population sizes (r=N_0/N_1) where r=1 signifies population stability, r<1 decline, and r>1 expansion. 4B-D) Posterior distributions of the current (N_0) and ancestral (N_1) effective population size using both the exponential (thick lines) and linear (thin lines) models: B) northwestern-historical, C) southern Sierra Nevada-contemporary, and D) southern Sierra Nevada-historical. The dotted line shows the prior distribution for N_0 and N_1.

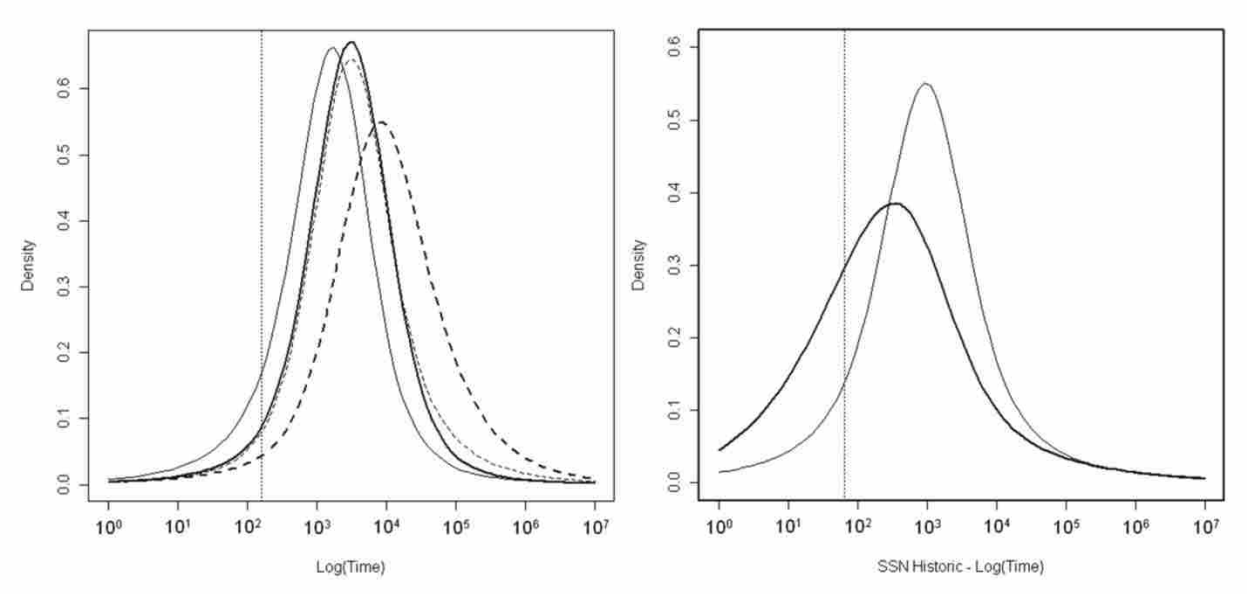

Figure 2-5. Posterior distribution of time of decline (*T*) for the linear (thin line) and exponential (thick line) models. A) Time (in years before present) for the contemporary SSN (solid lines) and NW (dashed lines). B) Time for the historical SSN. The vertical dotted line shows the approximate time of the European settlement of California (~1850) relative to the age of each of the samples.

Supplemental Tables

Table 2-S1. Location and collection date of historical fisher genetic samples. Samples were collected from the Smithsonian National Museum of Natural History (SNM) and the Museum of Vertebrate Zoology at the University of California, Berkeley (MVZ). Samples that successfully genotyped at a minimum of 7 of 10 microsatellite loci are shown in bold.

Population	Collection Date	Catalog Number	Location
Northwest	**5-Feb-1884**	**SNM-14395**	**Shasta County**
	1-Feb-1897	**SNM-87080**	**Cassel, Rock Creek Mountains**
	1-Feb 1897	SNM-87081	Cassel, Burney Mountain
	11-May-1889	SNM-30624	Cahto California, 3 mi S of Laytonville
	11-May-1889	SNM-24025	Cahto California, 3 mi S of Laytonville
	28-Mar-1905	MVZ-20955	Eden Valley
	1-Feb-1910	MVZ-12902	Helena
	20-Feb-1911	**MVZ-12901**	**Helena**
	13-Dec-1911	MVZ-16386	Head Ray's Gulch, 5 miles S of Cecilville
	19-Feb-1912	**MVZ-16531**	**Cecil Lake, 8 miles S of Cecilville**
	25-Mar-1912	**MVZ-16596**	**head Black Gulch, 10 miles S of Cecilville**
	27-Jan-1913	MVZ-19095	8 mi SE of Cecilville
	23-May-1917	SNM-227117	Covelo, California
	16-Sep-1917	SNM-227118	E. Fork Wells Creek, 15 miles E of Hayfork
	not recorded	SNM-21233	Shasta County
	not recorded	SNM-3415A	Fort Crooks , near Fall River Mills, California
Southern	**30-Jan- 1892**	**SNM-32315**	**Big Creek, Mariposa County**
Sierra	14-Jan-1893	SNM-51270	Big Creek, Mariposa County
Nevada	April-1893	SNM-52821	Wawona, Yosemite NP
	23-Dec-1895	**SNM-81094**	**Atwell's Mill, Sequoia NP**
	29-Mar-1905	MVZ-21396	Grouse Creek, Yosemite NP
	26-Jan-1911	SNM-171002	Yosemite Valley
	17-Dec-1915	MVZ-23668	Chinquapin, Yosemite NP
	28-Dec-1915	**MVZ-23883**	**6 miles S of Hetch Hetchy Valley**
	14-Jan-1915	**MVZ-23884**	**6 miles S of Hetch Hetchy Valley**
	20-Jan-1916	**MVZ-23885**	**6 miles S of Hetch Hetchy Valley**
	22-Feb-1916	MVZ-23686	Near Fort Monroe, Yosemite NP
	3-Feb-1917	**MVZ-24740**	**Fort Monroe, Yosemite NP**
	1-Dec-1918	**MVZ-29809**	**Grouse Creek, near Wawona Road, Yosemite NP**
	27-Jan-1919	MVZ-29810	Yosemite Valley
	28-Jan-1919	**MVZ-29811**	**Grouse Creek, near Wawona Road, Yosemite NP**
	1-Feb-1919	**MVZ-29812**	**Tuolumne Big Trees, Yosemite NP**
	1-Feb-1919	**MVZ-29813**	**Tuolumne Big Trees, Yosemite NP**
	1-Jan-1920	MVZ-31132	Hog Ranch Ranger Station (Mather), Yosemite NP
	1-Feb-1920	**MVZ-31133**	**Near Crane Flat, Yosemite NP**
	8-Feb-1920	**MVZ-31129**	**Near Big Meadows, Coulterville Rd, Yosemite NP**
	14-Feb-1920	**MVZ-31093**	**Yosemite Valley**
	14-Feb-1920	**MVZ-31094**	**Yosemite Valley**
	1-Apr-1920	**MVZ-31326**	**Yosemite NP**
	18-Jan-1919	**MVZ-29791**	**Yosemite NP**

Table 2-S2. Prior and hyperprior parameters for runs of the Storz and Beaumont (2002) analysis implemented in MSVAR. Columns 3-6 show the starting values for the mean and variance of the prior distributions. Columns 7-10 show the means and variances (and their means and variances) of the hyperprior distributions. Parameters listed are generation interval (g), current N_e (N_0), ancestral N_e (N_1), mutation rate (Θ), and time (T). All values are in a $\log_{10}$ scale.

Run#	g	log(N_0)	log(N_1)	log(θ)	log(T)	log(N_0)	log(N_1)	log(θ)	log(T)
01	5	4 1	4 1	-3.3 1	3 1	3 2 0 0.5	3 2 0 0.5	-3.3 0.25 0 0.5	3 2 0 0.5
02	5	4 1	4 1	-3.3 1	4 1	3 2 0 0.5	3 2 0 0.5	-3.3 0.25 0 0.5	3 2 0 0.5
03	4	4 1	3 1	-3.3 1	4 1	3 2 0 0.5	3 2 0 0.5	-3.3 0.25 0 0.5	3 2 0 0.5
04	5	3 1	3 1	-3.3 1	4 1	2 2 0 0.5	2 2 0 0.5	-3.3 0.25 0 0.5	3 2 0 0.5
05	5	4 1	4 1	-3.3 1	4 1	4 3 0 0.5	4 3 0 0.5	-3.3 0.25 0 0.5	3 2 0 0.5
06	5	4 1	4 1	-3.3 1	2 1	3 2 0 0.5	3 2 0 0.5	-3.3 0.25 0 0.5	2 2 0 0.5

CHAPTER 3: Sampling affects the detection of genetic subdivision and conservation implications for fisher in the Sierra Nevada

Abstract

The small population of fisher (*Pekania pennanti*) in the southern Sierra Nevada is completely geographically and genetically isolated putting it at increased risk of extinction. Previous research analyzing clustered samples found a high amount of genetic subdivision within the southern Sierra Nevada population hypothesized to be caused by the Kings River Canyon. In this study, we use a larger and more geographically continuous set of genetic samples (n=127) than was previously available to test this hypothesis of population subdivision by barrier and evaluate the genetic structure of this population. We found the population to be characterized by significant isolation by distance using 10 microsatellite loci. Both spatial and non-spatial population assignment models found three primary genetic clusters with moderate divergence between the clusters (F_{ST} = 0.05-0.13). These clusters appear to be associated with areas around the Kings River and Mountain Home State Demonstration Forest. One model also detected additional fine-scale subdivision north of the Kings River that may be evidence of founder effects from a recent population expansion. The amount of population subdivision detected in this study is lower than previously found and indicates that while certain landscape features may reduce gene flow, these landscape features may be less of a barrier than previously thought. We attribute the difference in results between this study and previous work to a difference in sampling. In the previous work, samples were collected in clusters that in populations exhibiting isolation by distance can inflate estimates of population structure by increasing the likelihood of oversampling related individuals. This study provides an empirical example of how clustered sampling of a continuously distributed population can affect the assessment of population subdivision and influence conservation implications.

Introduction

Conserving population connectivity is a conservation priority for many organizations interested in long-term species persistence (Crooks & Sanjayan 2006). Connectivity within and between populations is an important factor in many critical elements of population biology such as migration (Berger *et al.* 2008; Wilcove & Wikelski 2008), dispersal (Willson 2004; Kojola *et al.* 2009), spread of disease (Greer & Collins 2008; Plowright *et al.* 2011), and maintenance of genetic diversity (Epps *et al.* 2006; Dixo *et al.* 2009). Connectivity is especially important for small populations that are already at elevated risk of extinction due to their susceptibility to stochastic demographic or genetic factors (Gilpin & Soule 1986). Assessing the genetic structure of a population is a common method for evaluating connectivity by identifying boundaries of

genetic groups, quantifying the amount of gene flow between them, and distinguishing which landscape features might act to restrict gene flow (Perez-Espona *et al.* 2008).

The fisher (*Pekania pennanti* [Kopefli *et al*. 2008, Sato *et al.* 2012], formerly *Martes pennanti* [Erxleben 1777]) population in the southern Sierra Nevada is an example of a population for which concerns about connectivity are acute. The population is small, with an estimated size of less than 300 adults (Spencer *et al.* 2011), and isolated from the nearest native population by over 400 km (300 km to a recently reintroduced population). Recent research has shown that the southern Sierra Nevada fisher population has been genetically isolated from other fisher populations for thousands of years (Knaus *et al.* 2011; Tucker *et al.* 2012). Previous genetic analysis of this population found high amounts of subdivision ($F_{ST} = 0.51$) between two sampling areas north and south of the Kings River Canyon (Wisely *et al.* 2004). The two sampling areas were separated by less than 100 km, which is within the known maximum dispersal distance of the species (York 1996), of fairly contiguous forested habitat transected by the Kings River. The authors inferred that the Kings River could have been a barrier to gene flow between these two sampling areas. Detection of this strong subdivision raised conservation concerns that this small population was fragmented, furthering increasing extinction risk(Center for Biological Diversity 2008).

Recent research has found that estimates of structure and gene flow can be strongly influenced by sampling design depending on the genetic characteristics of the population (Novembre & Stephens 2008; Frantz *et al.* 2009; Schwartz & McKelvey 2009). For example a population may be distributed continuously across a landscape without barriers to gene flow which would result in discrete subpopulations. However, gene flow may be restricted to short distances, leading to increasing genetic differentiation as the geographical distance between individuals increases, termed isolation by distance (IBD,Wright 1943). IBD results in a gradient of genetic differentiation across a landscape where groups of individuals are genetically different, even though there is continuous genetic connectivity between them (Gonzalez□Suarez *et al.* 2009; Norén *et al.* 2011). In such populations, disentangling the effects of IBD from true ecological barriers is difficult and the impact of sampling design on results can be great. In particular, discontinuous sampling of a continuously distributed population characterized by IBD can lead to biased results where it appears that there is a genetic barrier on the landscape that is not really present (Schwartz & McKelvey 2009). For species of conservation concern understanding the effects of sample configuration on landscape genetic analyses and providing an accurate assessment of genetic structure is especially critical as this information can have a major impact in conservation decisions.

The Wisely *et al.* (2004) results are striking because of the high subdivision detected. However, this study was based on a small number of samples from two relatively limited geographic areas within the southern Sierra Nevada range (north Kings River n=14, south Kings

River *n*=19). If this population is characterized by IBD, such clustered sampling may have inflated estimates of population structure and the interpretation of that structure on the landscape. In this study, we assess the genetic structure of the southern Sierra Nevada fisher population using continuously distributed samples from across the entire population (Figure 3-1). We then test if sampling scheme (clustered versus continuous) strongly influenced the results.

Methods

Study Area

The study area is defined as the west slope of the Sierra Nevada south of highway 120 in Yosemite National Park to the southern tip of Greenhorn Mountains near Lake Isabella (Zielinski *et al.* 1995; Zielinski & Mori 2001). All sampling was conducted on federal lands and occurred within the known elevation range of fisher in this region (800- 3200 meters: Figure 3-1).

Sample Collection

Genetic samples were collected from 2006-2009. All sampling was conducted in conjunction with the U.S. Forest Service Sierra Nevada Carnivore Monitoring Program (Zielinski *et al.* 2012). 223 sample units were distributed across the study area and co-located with points from the Forest Inventory and Analysis (FIA) sampling grid. The FIA program is a nationwide forest condition monitoring program that consists of a sampling points located within a hexagonal grid network that are on average 5.47 km apart (Bechtold & Patterson 2005).

Each sample unit consisted of an array of 6 track-plate boxes with barbed wire hair snares that encompassed a 0.8 km^2 area (Zielinski *et al.* 2006). The use of bait (chicken) and a commercial trapping lure is thought to have extended the effective survey area of the sample unit to ~1.22 km^2 (Zielinski & Mori 2001). Positive identification of fisher from track data has been well established (Zielinski & Truex 1995) and only hair samples from stations that detected species within the genus *Martes* (fisher or marten) via tracks were genetically analyzed. Hair samples were collected and stored in prescription vials with a silica gel desiccant at room temperature to minimize DNA degradation.

Because we were concerned that gaps in sample distribution can bias analyses of population genetic structure, we also opportunistically deployed hair snares in Sequoia, Kings-Canyon, and Yosemite National Parks, as these areas were not included in the aforementioned sampling network. For this opportunistic sampling, track-plate boxes with hair snares were installed every 500-1000 meters along established roads or trails in areas. Stations were placed at least 50 meters from the edge of the road or trail to reduce any potential disturbance effects from close proximity to these anthropogenic features.

Laboratory Analysis

DNA was extracted from hair samples using the Dneasy Tissue Kit (Qiagen, Valencia, CA) with modifications for hair samples. Up to 10 hairs were used in each DNA extraction to maximize the probability of obtaining a high quantity of DNA. While the majority of genotypes (~70%) were obtained from field samples containing 5-10 hairs, a minority of genotypes were from samples consisting of 1-4 hairs. Samples were analyzed at ten microsatellite loci. *MP059*, *MP144*, *MP175*, *MP197*, *MP200,* and *MP247* were developed from tissue samples from the southern Sierra fisher population(Jordan *et al.* 2007). *MA1*, *GGU101, GGU216*, and *LUT 733* were developed in other mustelid species and have also been found to be variable in the Sierra fisher population (Davis & Strobeck 1998; Duffy *et al.* 1998; Dallas & Piertney 1998). The reaction volume (10 µl) contained 1.0µL DNA, 1x reaction buffer (*Applied Biosystems*), 2.0 mM $MgCl_2$, 200µM of each dNTP, 1µM reverse primer, 1µM dye-labeled forward primer, 1.5 mg/ml BSA, and 1U *Taq* polymerase (*Applied Biosystems*). The PCR profile was 94°C/5 min, [94°C/1 min, 55°C/1 min, 72°C/30s] x 36 cycles). The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). Hair samples that successfully genotyped were analyzed for sex using the Y-linked marker DBY-3 used successfully in wolverine (Hedmark *et al.* 2004). We tested DBY3 in fisher using samples of known sex and found this locus to consistently identify the correct sex for this species.

To address the potential for genotyping error from non-invasive samples, we used the multi-tubes approach in which each sample is analyzed a minimum of three times per locus with that locus accepted as accurate only if the samples produced consistent genotypes (Eggert *et al.* 2003; McKelvey & Schwartz 2004). If the genotype at a locus differed in one or more of these amplifications, we conducted an additional round of 3 amplifications. If a consistent genotype could not be determined after multiple amplifications then that locus was removed from the dataset. Samples that amplified at fewer than 7 loci were removed from further analysis.

We checked for genotyping errors using the examining bimodabilty (EB test) in the software DROPOUT (McKelvey & Schwartz 2005), which tests for bimodal peaks in the distribution of allele frequency differences among individuals that is indicative of genotyping error. We also used the program Micro-checker to assess the potential for null alleles at each locus (van Oosterhout *et al.* 2004).

Data Analysis

We tested microsatellite genotypes for departures from Hardy-Weinberg proportions at each locus using Fisher's exact test in Genepop 4.0 (Raymond & Rousset 1995; Rousset 2008). Gene diversity (H_E) (Nei 1973), F_{IS}, number of alleles (A), allelic richness (A_R) and gametic disequilibrium for each pair of loci were calculated using FSTAT 2.9.3 (Goudet 1995; Goudet 2001). The amount of genetic diversity present in the sample groups was compared using paired t-tests of arcsine-transformed H_E, and A_R (Archie 1985). We used sequential Bonferroni corrections to correct for multiple comparisons when assessing statistical significance (Rice

1989). We assessed the statistical power of the microsatellite panel to identify individuals by calculating the probability of identity (P_{ID}), the probability of two unrelated individuals randomly drawn from the population sharing the same multilocus genotype, using the method of Paetkau and Strobeck (1994). As shared ancestry and population subdivision can bias P_{ID} low we also calculated P_{SIB} which is the probability of two siblings sharing the same genotype. P_{SIB} defines the upper limit for the range of P_{ID} in a population when there is bias due to shared ancestry (Taberlet & Luikart 1999). Both P_{ID} and P_{SIB} were calculated in the software GenAlEx 6.4 (Peakall & Smouse 2006).

Spatial Autocorrelation

We tested for the presence of IBD using both Mantel tests of geographic versus genetic distance and spatial autocorrelation analysis in GenAlEx 6.4 (Peakall & Smouse 2006). We quantified spatial autocorrelation in the data by binning genetic distances between individuals into classes defined by geographic distance. We then visualized the data graphically using correlograms that illustrate the behavior of the autocorrelation as a function of distance (Manel *et al.* 2003).

To define statistical significance an autocorrelation coefficient representing no spatial structure was generated by randomly shuffling all individuals among geographic locations and calculating the autocorrelation coefficient over 1000 random permutations. Spatial autocorrelation was analyzed using 3 different geographic bin sizes of 4, 6, and 10 km to assess the effect of bin size on the results.

Population subdivision

Population structure was assessed using two individual based approaches that allow for the evaluation of genetic structure without defining *a priori* populations. Both methods used Bayesian analyses that generate genetic clusters by grouping individuals to minimize Hardy-Weinberg and linkage disequilibrium within groups. Individuals are then assigned probabilistically to the population from which its genotype most likely derived. One approach, implemented in the program STRUCTURE 2.3.3 (Pritchard *et al.* 2000), does not include geographic coordinates in its analysis, whereas the second approach, implemented in GENELAND (Guillot *et al.* 2005), incorporates specific spatial information for individuals in population assignments. We chose GENELAND as it has been shown to perform best for detecting boundaries, especially with semi-permeable edges (Safner *et al.* 2011). The spatial approach assumes that some degree of spatial dependence is present among individuals and that the probability of any two individuals belonging to the same population decreases with the geographical distance between them in accordance with Wright's isolation by distance model (Guillot *et al.* 2005).

In STRUCTURE we used a burn-in period of 100,000 iterations followed by a simulation length of 500,000 iterations, and allowed K to range from 1 through 10. We repeated the

analysis 5 times and averaged the resulting parameter values. We used an admixture model in which each individual has mixed ancestry and draws some fraction of its genome from each of the *K* populations, and ran both the independent and correlated allele frequency models (Pritchard *et al.* 2000; Falush *et al.* 2003). We also used the LOCPRIOR model (Hubisz *et al.* 2009) that incorporates location information by allowing for the *a priori* grouping samples based on sample location. The LOCPRIOR model has been found to improve inference for the true *K* when working with lower levels of divergence or less data compared to other STRUCTURE models.

To investigate the effect of the number of groups on the results we conducted 2 different LOCPRIOR analyses in STRUCTURE partitioning our data into groups of the 12-13 or 6-7 geographically closest individuals, and assigned each group an integer value resulting in 10 and 21 groups respectively. We determined *K* using two different methods: the maximum likelihood value ($\ln[\Pr(X|k)]$), and the ΔK method which is based on the second order rate of change of $\ln[\Pr(X|k)]$ between consecutive values of *K* (Evanno *et al.* 2005).

In GENELAND we conducted analyses using both the uncorrelated (similar to the independent model in STRUCTURE) and correlated allele frequency models. We chose to compare the results of both models because while the correlated model has been found to be more powerful at detecting subtle structure, it is also more sensitive to model assumptions and can overestimate *K* (Guillot *et al.* 2005). We used the spatial uncertainty option in GENELAND which allows for the locations of individuals to vary within a specified distance from the sample location and mimic the movement of an individual. This added variation addresses the problem of assigning individual genotypes to stationary points for mobile animals and has been found to increase the precision in detection of true population boundaries (Guillot *et al.* 2005). For individuals recaptured at more than one location we used the averaged UTM coordinates of all detections of that individual.

GENELAND simulations were conducted for 500,000 iterations with burn-in period of 40,000, maximum rate of Poisson process was set to 127 (the number of individuals), and the maximum number of nuclei set to 381 (3 times the number of individuals) as recommended by Guillot *et al.* (2005). Three different values of spatial uncertainty, 1000 m, 2000 m, and 5000 m were used to determine the influence of this parameter on the resulting population structure. *K* was set from 1 to 10 and each model was replicated 20 times. Models were ranked by the mean logarithm of posterior probability. We conducted post-process analysis on the top 3 runs of each model to visually assess consistency. The spatial domain was set to 100 and 200 pixels on the X and Y axes respectively.

For both STRUCTURE and GENELAND we used a cutoff probability (q-value) of 0.60 to infer population membership for each individual. We verified the F_{ST} values between

identified genetic clusters that are generated by STRUCTURE and GENELAND using Genepop 4.1 (Raymond & Rousset 1995; Rousset 2008) according to the method of Weir and Cockeram (1984). We also calculated an F_{ST} value between samples north and south of the Kings River to assess the *a priori* hypothesis, based on the Wisely *et al.* (2004) results, that the Kings River may be a major barrier to gene flow in the southern Sierra Nevada.

Results

Sample Collection and Laboratory Analysis

We successfully genotyped 247 hair samples from which we identified 127 unique genotypes representing different individuals. 85% of samples amplified at all 10 loci, 9% amplified at 9 loci, 3% at 8 loci and 3% at 7 loci. We detected more males (n=72) than females (n=48), and had 7 individuals for which we could not determine sex due to inconclusive results at the sexing locus. In the 42 individuals that were recaptured multiple times we detected fairly limited movements. The majority of both within year and between year recaptures occurred within the same sample unit or at other units in close proximity. We recaptured 12 individuals at more than one sample unit, with 11 of these 12 recaptures being male. The largest movement we found was a male that we detected in sample units 20 km apart over a 3 month time period.

Genetic Diversity

The 10 microsatellite loci had an average of 3.5 alleles/locus with an observed heterozygosity (H_O) of 0.51 and H_E of 0.56. The P_{ID} ranged from 0.00008 (7 loci) to 0.000002 (10 loci), and P_{SIB} from 0.01 (7 loci) to 0.002 (10 loci) which are within the values of 0.01-0.001 recommended for reliable identification of individuals (Waits *et al.* 2001). Two of the 10 loci were out of Hardy-Weinberg proportions, both of which had a significant deficit of heterozygotes (F_{IS} MP59 = 0.202, MP200 = 0.172), and were detected as potential null alleles by MICROCHECKER. Significant gametic disequilibrium was detected for 17 of the 45 loci pairs ($P < 0.05$), 15 more than the 2 that would be expected by chance alone. After correcting for multiple comparisons using a sequential Bonferoni correction, 3 loci pairs remained significant. However, all loci were in Hardy-Weinberg proportions and gametic disequilibrium after accounting for the population subdivision described in the following sections, indicating that the heterozygous deficit we detected was the result of underlying population subdivision (Wahlund 1928).

Isolation by Distance

The Mantel test of geographic versus genetic distance across all samples was highly statistically significant ($p<0.001$) indicating IBD, with a mantel correlation coefficient (r) of 0.25. The magnitude of this correlation is similar to or less than has been reported in other fisher population genetic studies (Kyle *et al.* 2001 r=0.75; Carr *et al.* 2007 r=0.38; Garroway *et al.* 2008 r=0.44; Hapeman *et al.* 20011 r=0.19). In estimates of spatial autocorrelation, the correlation coefficient r generally decreased across geographic distance throughout the population also indicating IBD (Figure 3-2).

Population subdivision

Bayesian clustering methods detected a moderate amount of subdivision within the southern Sierra Nevada fisher population. Both the independent and correlated models in STRUCTURE, found K=3 using the maximum ln[Pr($X|k$)] value, and K=2 using the ΔK method (Figure 3-3). The correlated model showed greater mixing among populations than the independent model.

Values of R, the parameter characterizing the amount of information in the *a priori* sample groups in the LOCPRIOR model, averaged 0.34 for the correlated model and 0.41 for the independent model. Small R values (<1.0) signify that the location data is informative to the model (Hubisz *et al.* 2009). The correlated and independent models in STRUCTURE had an average admixture value (α) of 2.54 suggesting a high degree of admixture as large α values (>1) indicate most individuals in the population are admixed. The mean α for K=3 (1.72) was much lower than for K=2 (3.35) indicating less admixture in individuals when the population was partitioned into 3 versus 2 populations. The variance in α over multiple iterations was also much lower for K=3 than K=2. Low variance in α has been found to be indicative of the true value of K (Prichard *et al.* 2000).

The uncorrelated model in GENELAND also found K=3 and this value of K was consistent across all 20 runs and all three levels of spatial uncertainty (Figure 3-4A). In both GENELAND and STRUCTURE for K=3 the identified genetic clusters roughly correspond to the area north of the Kings River (North), between the Kings River and the Mountain Home Demonstration State Forest (Central), and south of Mountain Home Demonstration State Forest (South) (Figure 3-5). Pairwise F_{ST} values between the clusters estimated in GENELAND were North-Central= 0.083, Central-South=0.054, North-South=0.127. Mantel tests for IBD within each of the North, Central, and South genetic groups were non-significant at α=0.05. This indicates that within each of these groups the genetic distance between individuals is independent of geographic distance. Testing the hypothesis of population division along the Kings River Canyon in Wisely *et al.* (2004), we found an F_{ST} value of 0.087 between samples groups north and south of the Kings River.

The geographic delineation of the clusters identified by STRUCTURE for K=3 were the same as found for K=3 in GENELAND. In STRUCTURE for K=2 the North and Central populations were combined into one larger population, separated from the South, with the boundary between populations nearly identical to K=3. The proportion of individuals assigning poorly to any one population was much greater for the STRUCTURE models than for the GENELAND models. In GENELAND 98.4% of individuals assigned with greater than a 0.60 probability to a population for K=3, while in STRUCTURE only 84.3% of individuals assigned at this probability threshold. Assignment rates remained high (90%) for GENELAND models

even when using at stricter 0.80 threshold for probability of population assignment, but dropped considerably (53.5%) for the STURCTURE models.

The correlated model in GENELAND, which has been shown to detect finer scale spatial structure than the uncorrelated model, found K=6 in the majority of simulations, with a minority of simulations finding K=5 (Figure 3-4B). The proportion of simulation showing K=5 decreased with increasing spatial uncertainty (1000 m = 20%, 2000 m = 10%, 5000 m = 5%). The correlated model showed similar genetic clustering as the uncorrelated model in the South and Central areas, but subdivided the North group into 4 smaller clusters (Figure 3-6). For the correlated model there were a greater number of individuals (n=6) that assigned poorly to one population, and all these individuals were within the North group. Pairwise F_{ST} values between the Central and the South group remained the same, but values between the Central group and the four North subgroups varied considerably ranging from moderate (0.042) to very high (0.169) (Table 3-1). The value of H_E was significantly lower at a 0.1 significance level in the North group than the Central and South. There was no significant difference between groups in A_R. (Table 3-2).

Discussion

Population subdivision in the southern Sierra Nevada

We found the southern Sierra Nevada population to have a moderate amount of genetic subdivision that appears to be associated with a number of geographic features or administrative areas including Little Shuteye Peak, the San Joaquin River, Kaiser Wilderness, Kings River Canyon and the Mountain Home Demonstration State Forest (Figure 3-6). The most consistently detected genetic clusters, found in both the STRUCTURE and GENELAND models, were 3 clusters with boundaries associated with the Kings River, and Mountain Home Demonstration State Forest. Results of this study contrast with Wisely *et al.* (2004) in the amounts of genetic subdivision; our results indicate areas of resistance to gene flow rather than major barriers. During the 4 year study period we detected very limited movement among recaptured individuals with the majority of recaptures either at the same or adjacent sample units, possibly indicating that long distance movements are relatively rare and that effective dispersal distance is likely much less than maximum dispersal capability (Kyle *et al.* 2001).

The highest levels of subdivision were found in a number of small genetic clusters north of the Kings River that were detected only by the correlated model in GENELAND. The strongest genetic clustering was north of Little Shuteye Peak (North-1: Figure 3-6) with that group showing F_{ST} values ranging from 0.137 to 0.164 between the 3 other northern genetic groups which were within 30 km of each other. It is possible that the additional genetic subdivision detected the North group is the result of multiple founder events within this area during a recent population expansion. The reduction in H_E in the North compared to the Central and South groups is consistent with the genetic signature expected to be generated by recent founder events (Nei *et al.* 1975, Hawley *et al.* 2006), but the lack of difference in allelic richness

between the groups is at odds with this hypothesis. However, survey data supports the idea of a recent population expansion. In the 1990's surveys routinely detected fisher in the central and southern portion of the study area, but rarely in the northern portion (Zielinski *et al.* 1995, 2005). From 1991-1994 fishers were detected only once at track-plate surveys in the Sierra National Forest (which encompasses the entirety of the North genetic group) whereas this study detected 44 individuals within this area. From 1996-2002 only 2 fishers were detected in Mariposa and Madera counties within the North genetic group (Zielinski *et al.* 2005), while this study detected 25 individuals from these same two counties.

Factors contributing to the assessment of population subdivision

We detected a much lower amount of population subdivision compared to Wisely et al. (2004), and did not find the Kings River Canyon to be a major barrier to gene flow. The main difference between the studies which likely accounts for the quantitative difference in F_{ST} values is the sampling design (see below). To a lesser degree, differences in markers likely played a role. We also consider other potential factors that could have contributed to the difference between these studies.

Sampling design

The difference in the results here and Wisely *et al.* (2004) is primarily attributable to 1) the geographic location of the samples available and 2) the possible sampling of close relatives. The samples used for the Wisely *et al.* (2004) study were from two relatively small and geographically disparate areas within the southern Sierra Nevada range (~98 km apart). While this distance is within the known maximum dispersal distance for fisher of 107 km (York 1996), it is much greater than the average dispersal distances recorded in other studies that ranged from 11-33 km (Arthur *et al.* 1993, York 1996, Aubry and Raley 2002). These two areas were on either side of the Kings River, which we found to be a possible source of genetic subdivision. In a population such as this, with IBD, we would expect clustered sampling to result in higher estimates of subdivision than a continuously distributed sample.

The majority of samples in Wisely *et al.* (2004) were from two very small areas: 12/14 samples in the north and 17/19 of the samples in the south were collected from within 87.1 km^2 and 49.9 km^2 areas respectively (minimum convex polygon area of sample locations). Considering home range sizes in the southern Sierra Nevada have been estimated at 5.3 km^2 (females) - 30.0 km^2 (males) (Zielinski *et al.* 2004) and are estimated to be more than twice as large in the northern portion of the southern Sierra Nevada fisher range (R. Sweitzer unpublished data) the Wisely *et al.* (2004) samples are likely an almost complete census of individuals within each area. Fishers have been shown to have male-biased dispersal and female philopatry with mean dispersal distances of males found to be 5 times greater than that of females (Aubry *et al.* 2004, Aubry and Raley 2006). Consequently, the dense sampling of individuals from these

small areas likely had a high degree of relatedness, especially among females, resulting in an inaccurate estimate of allele frequencies compared to the overall population.

We attempted to recreate the sampling scheme of Wisely *et al.* 2004 by subsetting the 2006-2009 samples to facilitate a direct comparison between the two studies. We selected an equivalent number of samples from the 2006-2009 dataset in the closest geographic proximity possible to the Wisely *et al.* (2004) samples as described in the previous paragraph. This resulted in much larger sampling areas of 223 km^2 (north) and 252 km^2 (south) and therefore, a much lower sampling density of individuals across both locations. Consequently, the differences in the spatial extent of sampling were so great that we felt that we could not meaningfully compare the two studies directly in this manner.

The influence of sampling design in the analysis of population structure has been addressed in a number of previous studies which have found that sampling can have a large impact on results (Rosenberg *et al.* 2005, Schwartz and McKelvey 2009, Frantz *et al.* 2009). When spatial autocorrelation exists in the population, sampling at a small scale will cause estimates of between group differentiations to be overestimated by minimizing within group variation and emphasizing between group variation (Schwartz & McKelvey 2009). To minimize this error, genetic samples should be representative of the entire population by including samples across the geographical range (Storfer *et al.* 2006).

Sample size

The sample size required to precisely estimate F_{ST} has been found to be dependent on the amount of subdivision present (Kalinowski 2004; Yang *et al.* 2005). The lower the amount of subdivision the greater the number of samples required, such that very large sample sizes are only required when the amount of subdivision between populations is very low (Kalinowski 2004). Using a simulated dataset with a suite of 16 loci, Kalinowski (2004) found that 100 individuals per population would be needed for precise estimation if F_{ST} =0.01, but only 20 individuals per population would be sufficient if F_{ST} =0.05. Considering that the amount of subdivision detected between genetic groups in this study was moderate (0.054-0.127) and high (0.51) in Wisely *et al.* (2004), the smaller sample size used by Wisely *et al.* (2004) does not likely explain the difference in results. Therefore, the discrepancy in sample sizes between this paper (n=127) and Wisely *et al.* (2004) (n=33) is not likely driving the difference in F_{ST} estimates.

Genetic diversity of loci

The amount of genetic variation within loci can influence estimates of subdivision. The maximum F_{ST} value is directly related to the heterozygosity (H_E) of markers such that the maximum $F_{ST} = 1 - H_E$. Consequently, when using loci with higher heterozygosity the maximum possible F_{ST} value is lower. The 8 loci examined by Wisely *et al.* (2004) had extremely low

variability in the southern Sierra Nevada fisher population, with H_E values of 0.16 and 0.20 north and south of the Kings River respectively. This low variation is likely the result of the loci being developed for use with stoats (*Mustela ermina*) and martens (*Martes americana*) because microsatellites specific for fisher were not yet available. If loci are developed in other species then there is the potential for ascertainment bias such that loci that are highly polymorphic in one species tend to have less diversity in related species (Ellegren *et al.* 1995; Cooper *et al.* 1998). Additionally, because some of these 8 loci were fixed in the Sierra Nevada populations, the overall power to precisely describe genetic subdivision was further reduced.

In 2007, a new set of microsatellite loci were developed using fisher tissue from the southern Sierra Nevada (Jordan *et al.* 2007); this resulted in 6 new polymorphic loci for the study population which have a much higher diversity than in the loci previously available. The lower F_{ST} detected in this study could result from the increased heterozygosity of the markers (H_E = 0.56). However, while the 10 loci used in this study showed higher heterozygosity and allelic richness than the Wisely *et al.* (2004) loci, overall genetic diversity was still low. The low diversity may result from the small size of the population, its long term isolation (Knaus *et al.* 2011; Tucker *et al.* 2012), and its biogeographic history (Wisely *et al.* 2004) all of which likely contributed to the loss of genetic diversity over time. We calculated G'_{ST} according to Hedrick (2005) which standardizes F_{ST} by sample heterozygosity. The estimates of divergence were still very different (G'_{ST} [Wisely *et al.* 2004] = 0.76, G'_{ST} [this study] = 0.31) and thus differences in heterozygosity of the loci used do not wholly explain the difference in the results.

Considerations for population subdivision analyses

Our data showed a significant IBD pattern with genetic distance significantly correlated with geographic distance across the study area. IBD has been found to have a confounding influence in landscape genetic analyses (Manel *et al.* 2003; Musiani *et al.* 2007). Pritchard *et al.* (2000) acknowledged that STRUCTURE is not well suited to analysis in the presence of IBD. The spatially based analysis in GENELAND has similar assumptions as the IBD model, that geographically close individuals are more likely to be related, and so may be better suited for this analysis. This may explain why the STRUTURE models had a greater proportion of individuals that assigned poorly to a population compared to the GENELAND models.

While the ΔK and maximum likelihood methods in STRUCTURE yielded different values of K = 2 and K=3 a number of factors indicate that K=3 is more indicative of the true K. We found a lower mean α for K=3 indicating individuals assigned with more certainty to each population than when K=2. The variance in α was much lower for K=3 than K=2 which, according to Pritchard *et al.* (2000), is indicative that K=3 better represents the true population structure. Additionally, the STRUCTURE population assignments for K=3 agreed with those from the uncorrelated GENELAND model which also found K=3.

An important consideration in attributing population subdivision to landscape features is consideration the temporal dynamics between the two factors. Landguth *et al.* (2010) found there were lag times between either the establishment or removal of a genetic barrier and the detectability of the resulting genetic structure. Depending on the dispersal and movement characteristics of a species it can take tens to hundreds of generations until the genetic data reflects either the appearance or loss of a barrier. Consequently, it can be difficult to discern whether the observed subdivision is due to historical or contemporary landscape elements. This problem is minimized for species with relatively long distance dispersal. Landguth *et al.* (Landguth *et al.* 2010) found using simulations that it takes less than 10 generations to lose 50% of the barrier signal if maximum dispersal distance is >30 km. Considering the maximum recorded dispersal distance for fisher is ~100 km (York 1996) the population subdivision observed in this study is likely attributable to relatively recent landscape conditions rather than historical conditions. However, considering fisher's generation interval is ~ 5 years, the observed structuring may be the result of landscape conditions over the last few decades rather than current conditions.

Conclusions

We found that the southern Sierra Nevada fisher population is not characterized by high subdivision as previously thought. Overall, fisher subdivision in the southern Sierra Nevada follows a pattern of IBD with additional structuring that corresponds to geographic features and management boundaries. This landscape can be characterized as having areas that are resistant to gene flow but without major barriers. However, the limited movement distances we found among individuals recaptured across multiple years suggest that long distance movements may be uncommon in this population. We found the genetic subpopulations to be connected by moderate amounts of gene flow that may actually help to counteract the effects of genetic drift due to small population size and help maintain genetic diversity with the southern Sierra Nevada population over time. Perhaps most importantly, this study provides an empirical example of the influence that sampling can have on population genetic analyses.

While the magnitude of subdivision we detected was different between studies, both studies found that gene flow in fishers is sensitive to landscape features. Maintaining connectivity within this population will rely on determining what specific landscape elements are acting to restrict gene flow within the population. Identifying these landscape features is critical to prevent creation or expansion of anthropogenically influenced landscape elements that may further restrict gene flow and to plan for shifts in connectivity due to predicted changes in the landscape from climate change. Future planned research will use an individual based genetic approach in a resistance modeling framework (McRae *et al.* 2008; Garroway *et al.* 2011; Sawyer *et al.* 2011) to ascertain what specific landscape features may be creating the observed genetic subdivision by impeding or facilitating gene flow.

The comparison between these two studies, and the realized differences between their results and associated conservation implications, provides a clear example of the need to reassess other early genetic studies for species of conservation concern. Technological advances in laboratory analysis make marker development less expensive and easier, and the advent of new analytical methods in the emerging field of landscape genetics (Manel *et al.* 2003) allow for more quantitative analyses of landscape features. These advances, combined with the ability to noninvasively collect genetic samples of populations on a large scale, allow the next generation of conservation genetic work to obtain a clearer picture of the population structure of a species than was previously possible. As this comparative study shows, such a reexamination can have important implications in the management and conservation of a species.

Acknowledgments

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Table 3-1. F_{ST} values for the population assignments identified in the GENELAND correlated frequencies model.

	North - 1	North - 2	North - 3	North - 4	Central
North - 1	-				
North - 2	0.137	-			
North - 3	0.164	0.023	-		
North - 4	0.138	0.079	0.055	-	
Central	0.169	0.096	0.042	0.056	-
South	0.198	0.104	0.074	0.137	0.054

Table 3-2. Expected heterozygosity (H_E), F_{IS}, and allelic richness (A_R), between the North, Central, and South genetic groups and the total population.

Group	n	H_E	F_{IS}	A_R
North	44	0.474	0.043	2.78
Central	32	0.552	0.061	2.80
South	51	0.561	0.024	2.95
Entire SSN	127	0.565	0.101	3.04

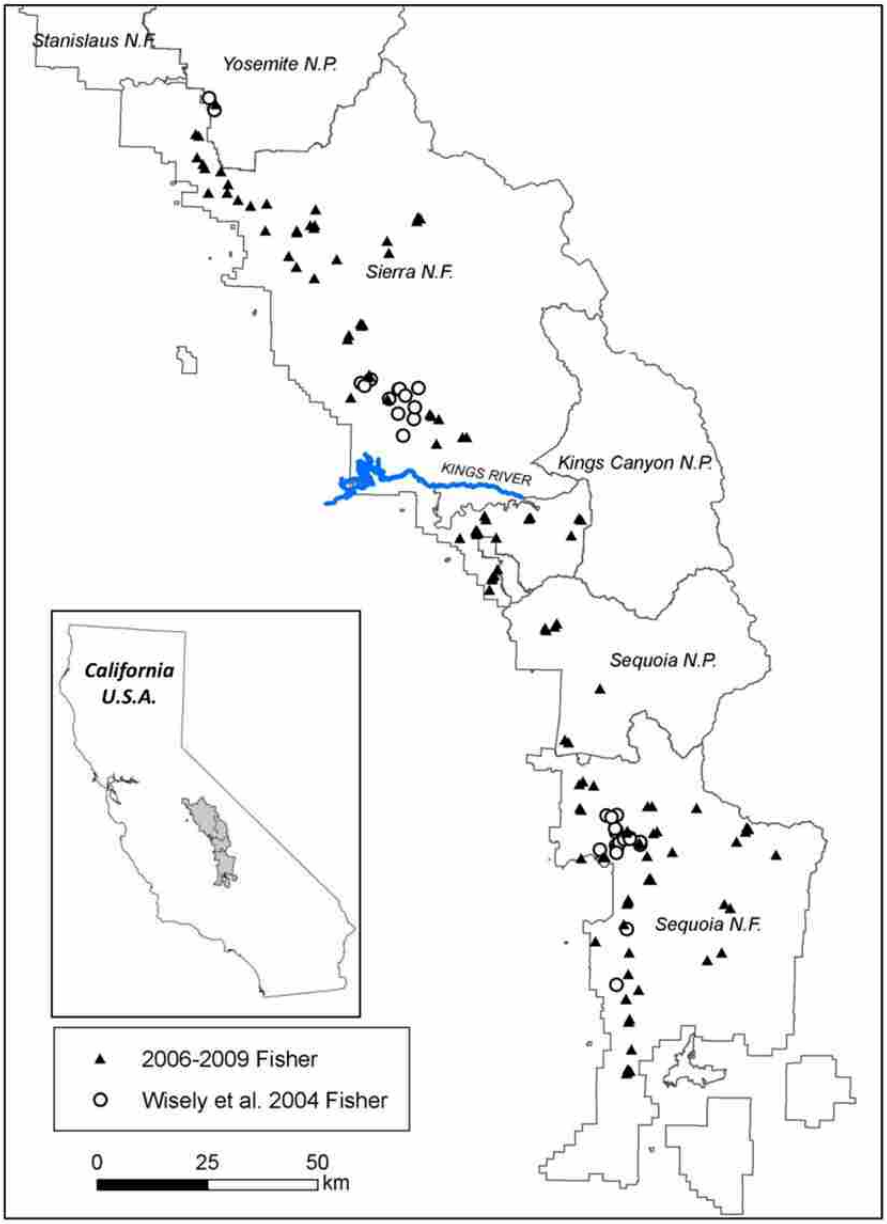

Figure 3-1. Location of the southern Sierra Nevada fisher population and the distribution of samples from Wisely *et al.* (2004) (open circles) and samples from the 2006-2009 sampling for this study (black triangles)

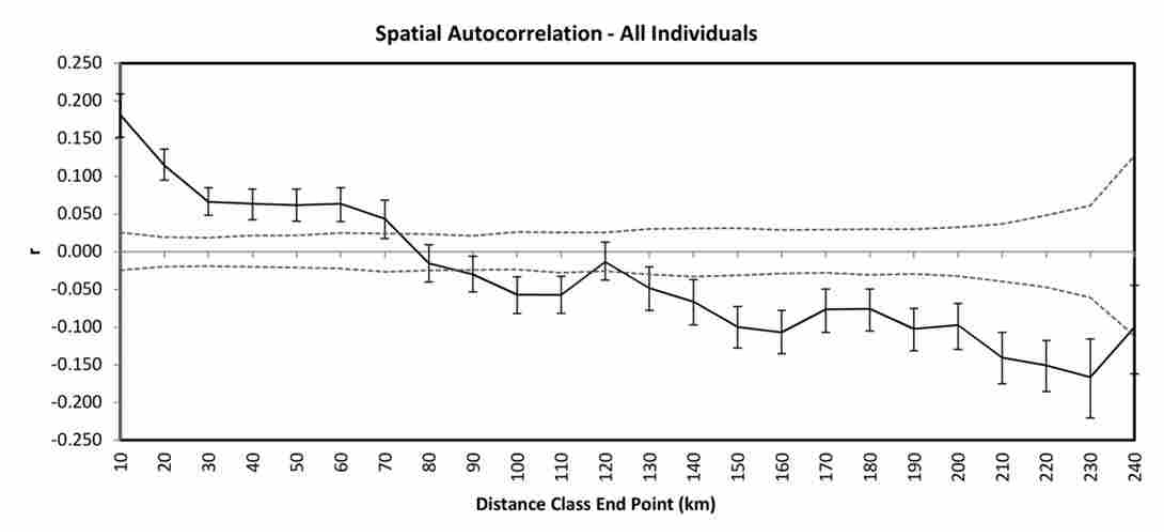

Figure 3-2. Correleogram showing the results of spatial autocorrelation analysis with the correlation coefficient *r* (solid line) as a function of distance (10 km bin size) with 95% confidence interval error bars and a 95% confidence interval (dotted line) around the null hypothesis of no spatial structure

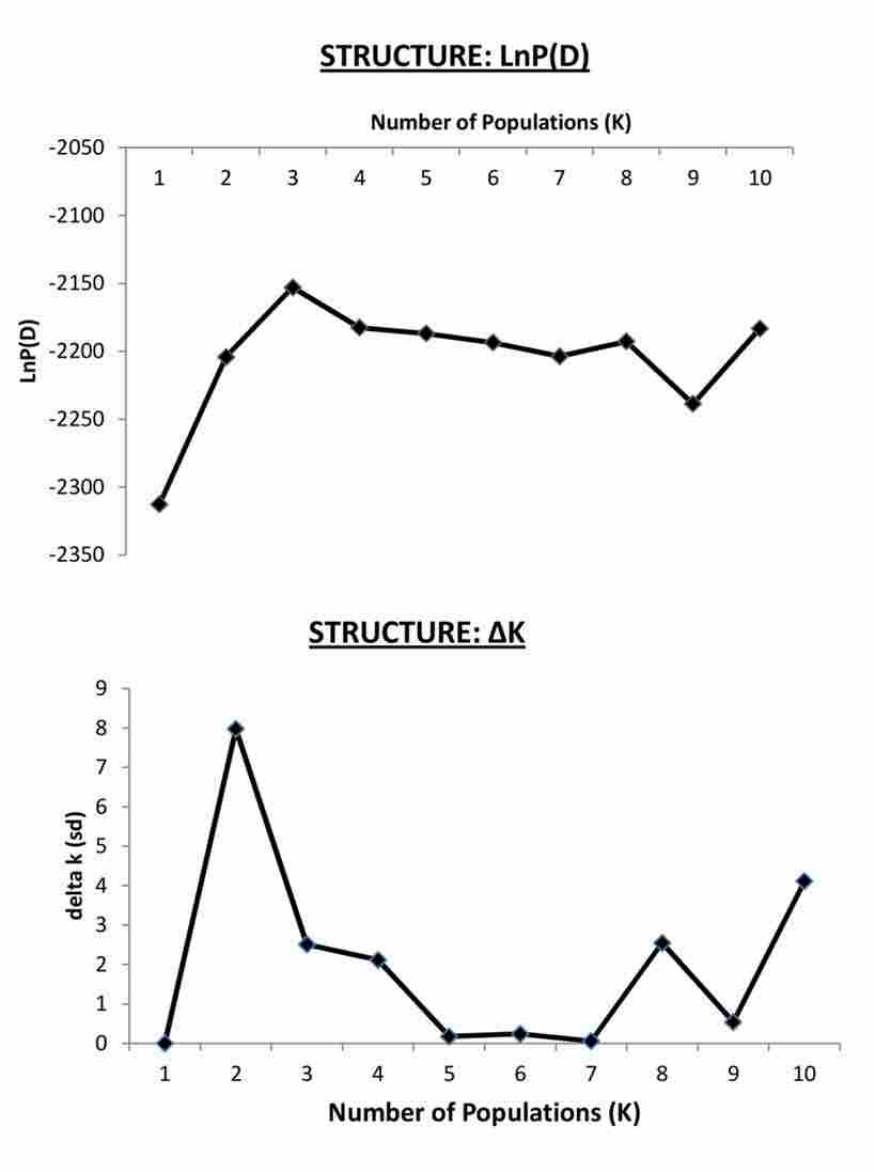

Figure 3-3. Results of the admixed, correlated model in STRUCTURE showing the modal value of K=3 for the maximum likelihood method and K=2 for the ΔK method. Results were similar for the admixed independent and LOCPRIOR models.

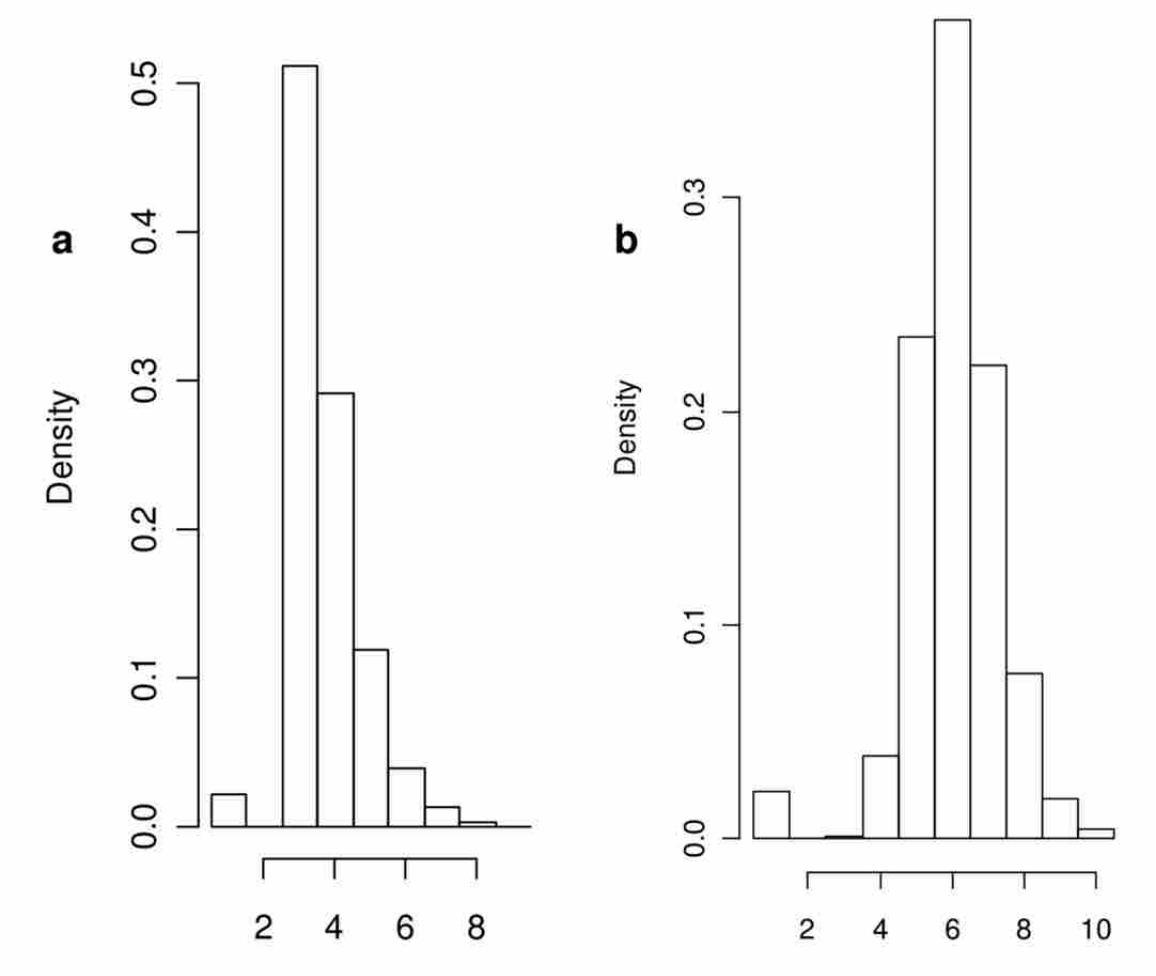

Figure 3-4. Histogram of the distribution of simulation results over 500,000 iterations for the number of populations (*K*) for the GENELAND A) uncorrelated and B) correlated models.

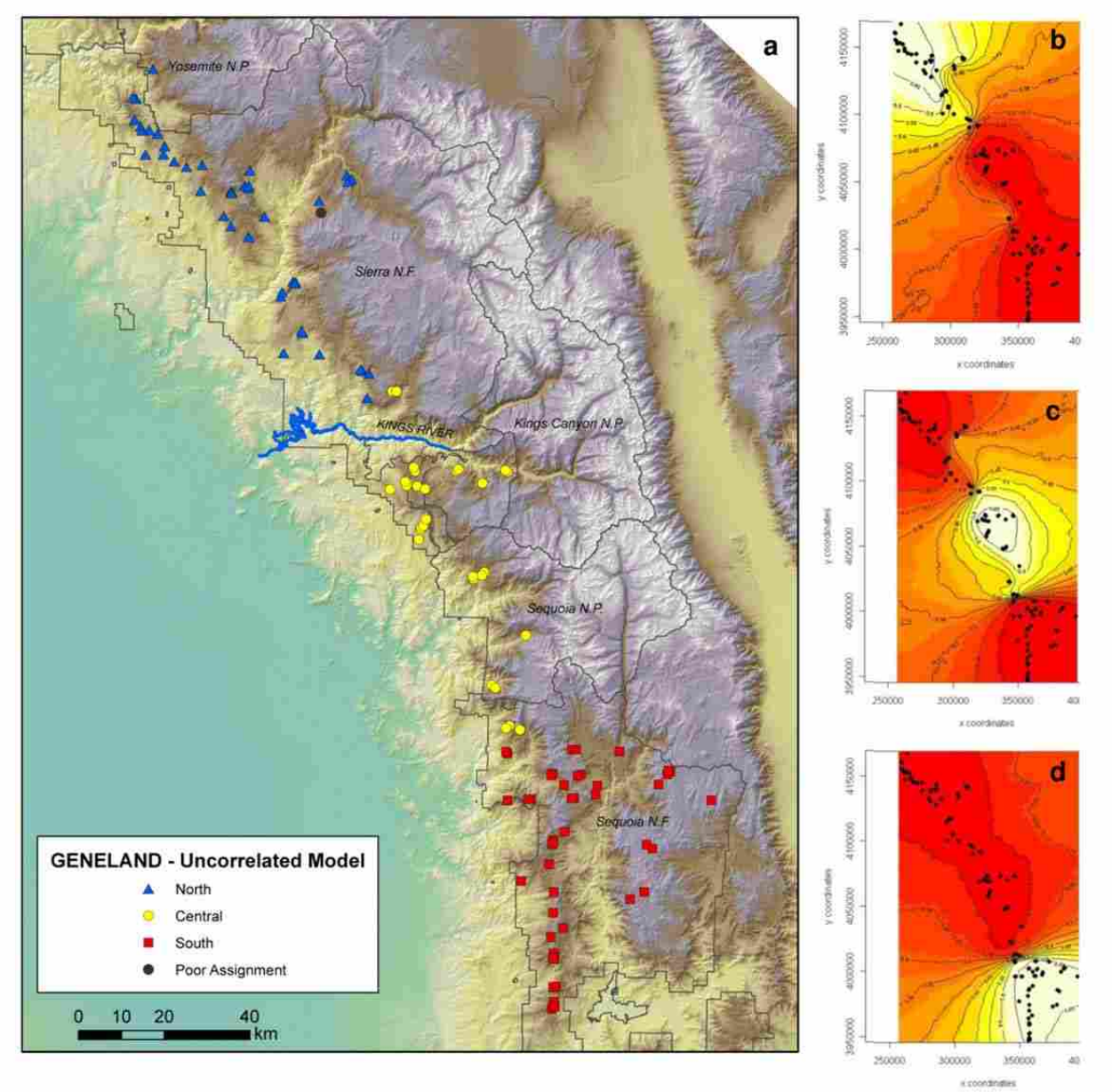

Figure 3-5. a) Map showing the assignment of individuals to each of the identified genetic clusters using GENELAND for the uncorrelated model (*K*=3). Individuals that assigned poorly to any one population are shown in black. b-d) Maps showing the posterior probabilities of cluster membership to b) North, c) Central, and d) South genetic groups. Individuals in areas with white shading indicate fisher that had a high probability of assignment to that genetic group.

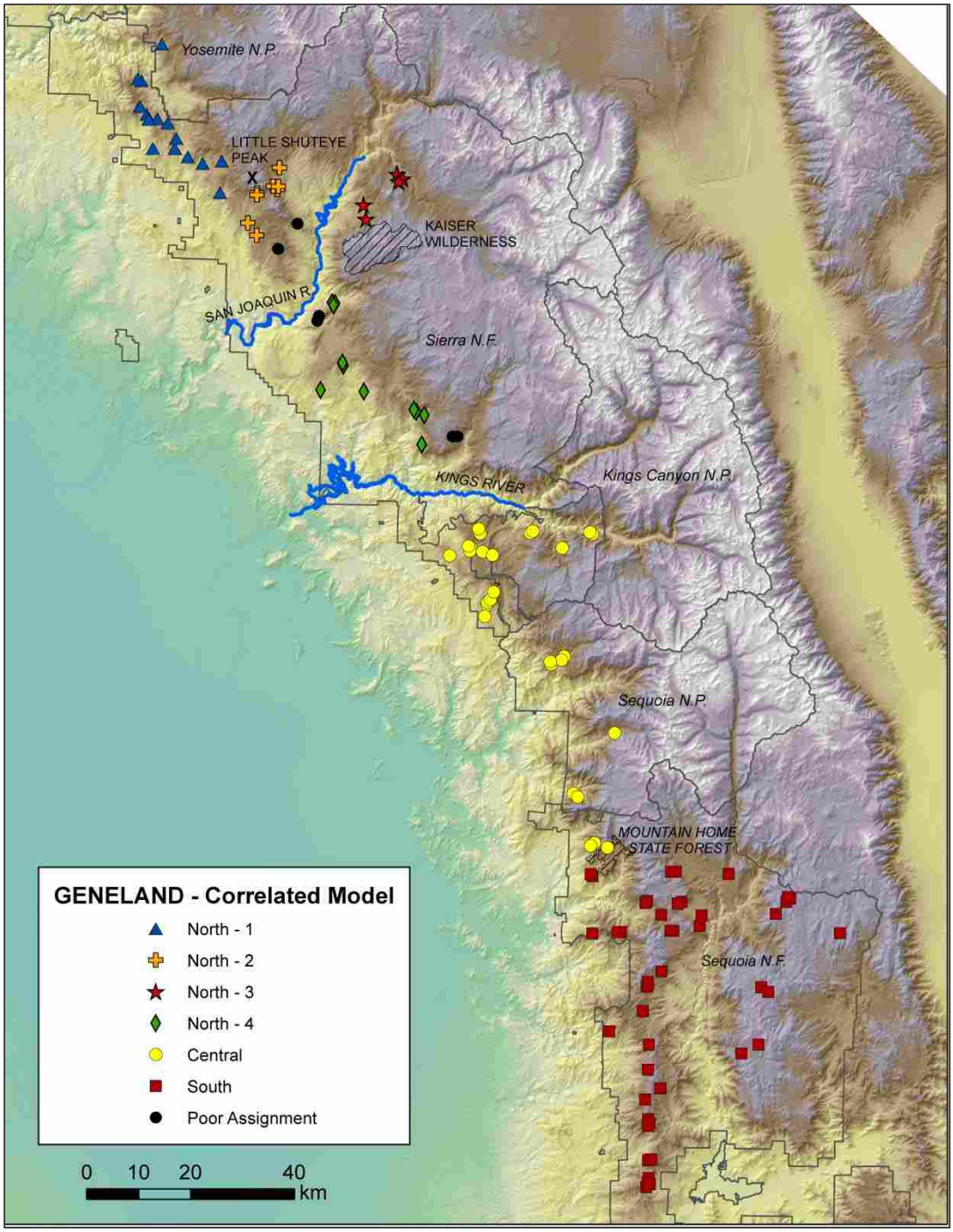

Figure 3-6. Map showing the assignment of individuals to each of the identified genetic clusters (*K*=6) using the correlated model in GENELAND. Individuals that assigned poorly to a population are shown in black.

CHAPTER 4: The effect of sex-biased dispersal and spatial heterogeneity on modeling landscape resistance to gene flow

Abstract

Genetic connectivity results from the dispersal and reproduction of individuals across a landscape. Genetics analysis provides a method to assess dispersal indirectly as it is often difficult to directly estimate dispersal at large spatial scales. Mammalian populations frequently exhibit sex-biased dispersal, with males dispersing longer distances than females, but this factor has rarely been addressed in landscape genetics research. In this study, we model the effects of landscape features and sex-biased dispersal on gene flow for a small and isolated population of fisher (*Pekania pennanti*) in the southern Sierra Nevada Mountains of California. We genotyped 127 fishers at 10 microsatellite loci, and determined sex with a Y-linked marker. We found that females have a significantly higher genetic divergence (F_{ST}) between subpopulations than males. We also found a tendency for an excess of homozygotes within subpopulations for males ($F_{IS} > 0$), but not for females. Both of these differences between sexes are expected if males disperse more than females. We developed resistance surfaces from 8 landscape features that we hypothesized to affect gene flow. We used multiple regression of distance matrices to fit models of genetic distances to resistance distances for both sexes and in multiple geographic areas. Using model selection, we show that sex-biased dispersal and landscape heterogeneity affected the determination of what landscape features structure gene flow. We found that for females gene flow was impeded by major water bodies and roads and facilitated by mid-elevation dense forests, with water having the strongest effect in the North subpopulation and roads the strongest effect in the South subpopulation. We found gene flow for males was impeded by steep slopes and facilitated by dense forest over a wide elevation range. However, model fit for males was markedly lower than for females, indicating that landscape features have comparatively little influence on male gene flow. Our results suggest that careful consideration of the potential for sex-biased dispersal, and landscape heterogeneity should be undertaken prior to conducting landscape genetic analyses because these factors can strongly influence results.

Introduction

Connectivity, defined as the ability of organisms to move within and among populations, is fundamental for long-term species persistence (Lowe & Allendorf 2010). For small populations vulnerable to stochastic events, demographic and genetic connectivity is essential for maintaining population viability (Gilpin & Soule 1986). Genetic connectivity results from the successful dispersal and reproduction of individuals across a landscape. Therefore, understanding the dispersal characteristics of a species and gene flow are inherently intertwined. Estimating dispersal through direct methods (mark-recapture, telemetry) is difficult for many species as it can be cost prohibitive to capture and track many animals over large landscapes.

This is especially true for rare or difficult to detect species. Direct methods also cannot easily distinguish between movement of an animal and gene flow, in which movement is followed by successful reproduction. Genetic methods provide an alternate suite of methods for assessing dispersal (Slatkin 1985). These genetic methods, when coupled with the use of non-invasive genetic sampling that can cost-effectively sample individuals over large areas, provide a powerful tool by which we can improve our understanding of dispersal across landscapes.

The dispersal of an individual will be shaped by its ability to move through the landscape and consequently, landscape features that influence dispersal will also influence gene flow. Using genetic information to discern what landscape features facilitate or impede dispersal is field of research termed landscape genetics (Manel *et al.* 2003). Gene flow can be influenced by a wide variety of factors including abiotic factors such as topography (Murphy *et al.* 2010), climate (Schwartz *et al.* 2009), or anthropogenic features (Blair *et al.* 2013; Epps *et al.* 2013) and biotic factors such landcover type (Cushman *et al.* 2006), predation (Murphy *et al.* 2010), or prey availability (Sonerud *et al.* 1988).

Sex-biased dispersal is a well-documented characteristic of many wildlife populations (Pusey 1987). In mammalian species dispersal is often male biased (Dobson 1982) whereas in birds dispersal is usually female biased (Clarke *et al.* 1997; but see Pierson *et al.* 2010). There are many hypotheses as to why sex-biased dispersal occurs, including resource competition (Greenwood 1980), inbreeding avoidance (Pusey 1987), and local-mate competition (Dobson 1982). In polygynous mammals females usually have a larger parental investment (gestating, nursing, feeding) than males, and therefore mating competition is much stronger for males than females (Dobson 1982). This results in males dispersing longer distances from their natal area than females to avoid competition and find unoccupied territories, whereas, females tend to establish home ranges close to their natal area (Greenwood 1980; Dobson 1982). However, while sex biased dispersal is a common characteristic or mammalian populations it is not universal (Favre *et al.* 1997, Boyd and Pletscher 1999). Understanding sex-biased dispersal is an important factor in conserving population connectivity, as factors important to maintaining connectivity for the dispersing sex may differ from those important to the more philopatric sex. and effective conservation strategies must address these sex specific differences. Assessing sex-biased dispersal using genetic methods is well established (Goudet *et al.* 2002; Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007; Clutton-Brock & Lukas 2012).

The influence of landscape features on dispersal can also vary spatially. This may be due to biological variation within a population, such as variable dispersal pressure due to heterogeneity in the density of individuals (Matthysen 2005), interspecific interactions (Rundle & Nosil 2005), or due to the differential availability of a feature on the landscape. Short Bull *et al.* (2011) identified the importance of replicating study areas in landscape genetics, and found that the landscape features influencing gene flow in American black bears varied among study

areas due to the variability of the landscape feature in each area. For example, in some of their populations elevation was constant and had no relationship with gene flow, while in other areas elevation varied substantially and was found important for explaining gene flow. This conclusion has been found in a number of subsequent landscape genetic analyses (Moore *et al.* 2011; Trumbo *et al.* 2013).

In this study, we examine how sex-biased dispersal and landscape heterogeneity affect gene flow in a small and isolated population of fisher (*Pekania pennanti* [Kopefli *et al*. 2008, Sato *et al.* 2012], formerly *Martes pennanti* [Erxleben 1777]) in the southern Sierra Nevada Mountains of California. Identifying the landscape elements structuring genetic connectivity and dispersal for the southern Sierra Nevada population of fishers has important conservation implications. Due to its small population size, estimated at <300 adults (Spencer *et al.* 2011), and long-term genetic isolation (Knaus *et al.* 2011; Tucker *et al.* 2012) there are acute conservation concerns regarding the long-term viability of this population which is currently a candidate for listing under the federal Endangered Species Act (U.S. Department of the Interior, Fish and Wildlife Service 2004).

The southern Sierra Nevada population of fishers provides a useful study system to address questions of sex-biased dispersal and landscape heterogeneity for a number of reasons. Sex-biased dispersal has been documented in other fisher populations (Kelly 1977; Powell 1993; Aubry *et al.* 2005) and in related mustelid species (Zalewski *et al.* 2009; Vangen *et al.* 2001) and so we hypothesized that this is likely a feature of the Sierra Nevada fisher population as well. Secondly, the southern Sierra Nevada fisher population occupies a large and diverse landscape that has potential for spatial variation across the study area. A previous study has identified three genetic subpopulations within the southern Sierra Nevada (Chapter 3, Tucker *et al.* In Review) that can be used to define groups for replication of landscape genetic analyses (Figure 4-1). Thirdly, fisher habitat selection has been well studied within California (Zielinski *et al.* 2004; Davis *et al.* 2007; Spencer *et al.* 2011) and there have been a number of other genetic studies identifying landscape features influential for gene flow in fishers in other locations throughout their range (Carr *et al.* 2007; Garroway *et al.* 2008; Garroway *et al.* 2011; Hapeman *et al.* 2011) providing a diverse suite of candidate variables from which to test hypotheses regarding landscape influence on gene flow.

In this study, we used fisher genetic samples from across the southern Sierra Nevada to ask the following questions: 1) what landscape features influence genetic connectivity in this population, 2) do these landscape features vary by sex due to sex-biased dispersal, and 3) do these landscape features vary spatially across the study area? We investigated these questions using a resistance modeling approach in which we selected landscape variables for which we had expectations of their relationship with gene flow. We generated resistance surfaces representative of our hypothesized relationships. For each resistance surface we used circuit

theory (McRae 2006) to estimate the resistance distance between individuals. We then fit models of pairwise resistance distance between individuals to pairwise genetic distance using multiple regression on distance matrices (MRDM) (Legendre *et al.* 1994), and used a model selection approach to identify the best supported model (Burnham & Anderson 2002). To test our hypotheses that sex-biased dispersal and landscape heterogeneity affect fisher gene flow we conducted resistance-modeling analyses separately for both sexes, and in multiple geographic areas. We used the resulting model parameters to create multivariate resistance surfaces by sex for the overall study area and for each geographic subset.

Methods

Study area, sample collection, laboratory analysis, population genetic metrics

Details regarding the study areas, sampling methods, laboratory analysis and calculation of basic population genetic statistics are detailed in Chapters 2 and 3 (Tucker *et al.* 2012, Tucker *et al.* In Review). Genetic samples were collected from 2006-2009 in conjunction with the U.S. Forest Service Sierra Nevada Carnivore Monitoring Program (Zielinski *et al.* 2013). Hair snares were deployed at 223 sample units distributed across the fisher range in the southern Sierra Nevada. Each sample unit consisted of an array of 6 track-plate boxes with barbed wire hair snares that encompassed a 0.8 km^2 area. We also opportunistically deployed hair snares in Sequoia, Kings-Canyon, and Yosemite National Parks to fill in gaps in the sample distribution as these areas were not included in the aforementioned sampling network for the monitoring program. We used 10 microsatellites (Dallas & Piertney 1998; Davis & Strobeck 1998; Duffy *et al.* 1998; Jordan *et al.* 2007) to genotype 247 spatially referenced fisher samples. The 10 microsatellite locus panel averaged of 3.5 alleles/locus with an H_E of 0.56 and F_{IS} of 0.10.

Sex-biased dispersal

We tested our hypothesis of sex-biased dispersal using a series of genetic analyses that test for differences between males and females in 2 different genetic metrics: F_{ST} and the assignment index (AI) (Goudet *et al.* 2002). The AI estimates the probability of each individual's genotype originating within its geographic population of capture such that negative values (<0) indicate potential dispersers and positive values (>0) indicate resident individuals. With sex-biased dispersal we would expect the dispersing sex, in this case males, to have a lower mean AI and higher variance of AI compared to the philopatric sex (Goudet *et al.* 2002). For the F_{ST} test we would expect the philopatric sex to have significantly higher F_{ST} values between subpopulations than the dispersing sex. Sex-biased dispersal tests were conducted using Fstat 2.9.3 (Goudet 2001). We also estimated F_{IS} by sex for each subpopulation using Genepop 4.2 (Raymond & Rousset 1995; Rousset 2008). The dispersing sex should have a positive F_{IS} caused by a Wahlund effect because the dispersers' genotypes originated in a different subpopulation from where they were sampled (Wahlund 1928).

We also further assessed the population structure identified by Tucker *et al.* (In Review) by conducting individual based population assignment analyses for males and females independently. This sex-based population assignment analysis was conducted in R version 2.15.2 (R Core Team 2012) using the package GENELAND version 4.0.3 (Guillot *et al.* 2005). In GENELAND we conducted analyses using the uncorrelated model implementing the spatial uncertainty option which allows for the locations of individuals to vary within a specified distance from the sample location and mimic the movement of an individual. Analyses were conducted for 500,000 iterations with burn-in period of 40,000, maximum rate of Poisson process was set to 127 (the number of individuals), and the maximum number of nuclei set to 381 (3 times the number of individuals) as recommended by Guillot *et al.* (2005). Spatial uncertainty in coordinates was set to 2000 m and *K* was allowed to vary from 1 to 10. Each model was then replicated 20 times. Models were ranked by the mean logarithm of posterior probability. We conducted post-process analysis on the top 3 runs of each model to visually assess consistency. We also assessed sex-biased dispersal using a landscape resistance approach by analyzing resistance models for males and females separately (details below).

Defining resistance landscapes

We identified 8 landscape features for which we had *a priori* hypotheses regarding how they may affect gene flow based on a combination of variables found important in previous studies for fisher occupancy, home range composition, and gene flow (Table 4-1). There were 6 landscape features that we hypothesized to be resistant to genetic connectivity including major water bodies (rivers and lakes) (Wisely *et al.* 2004; Garroway *et al.* 2011), roads (Garroway *et al.* 2011), steep slopes (Jordan 2007), open areas (Powell 1994), moderate or high severity fire areas (Scheller *et al.* 2011), and both very low (<915 m) and very high (> 2440 m) elevations (Davis *et al.* 2007; Spencer *et al.* 2011).

We also hypothesized that areas with large trees and high canopy cover would facilitate gene flow (Zielinski *et al.* 2004; Purcell *et al.* 2009) and included two related variables reflecting this hypothesis. Both of these variables are based on habitat types, tree class sizes, and canopy density defined by the California Wildlife Habitat Relationship classification system (Salwasser *et al.* 1980) and included forest types with large trees (>28 cm) and dense canopy cover (>60%). The two variables differ in the presence of the Sierra Mixed Conifer habitat class. The exclusion of this habitat class from one variable (dense forest) restricted the elevation range of the forested pixels to the core of the occupied fisher range in the Sierra Nevada (1400-2300m) (Spencer *et al.* 2011), whereas its inclusion in the second variable (Sierra Mixed Conifer) expanded forested areas to margins of the fisher elevation range. These higher elevations areas may not be optimal habitat for fisher occupancy, but we hypothesized that they may play an important role in fisher gene flow.

Resistance layers were generated using ArcGIS 10 (ESRI 2011) using a pixel size of 100m. All variables were coded as resistance surfaces with pixels hypothesized to facilitate gene flow assigned a value of 1 and pixels resistant to gene flow assigned a value greater than 1. As map edges have been shown to bias resistance values (Koen *et al.* 2010) we buffered all fisher locations by a minimum of 12 km. For individuals captured multiple times we assigned the individual the coordinates of its initial detection. The calculation of resistance distances requires only one individual be located in pixel, if two individuals occupied the same pixel we moved one of them ~100 m in a random direction into a different pixel. Because our sampling method involved baited stations and scent lure that potentially draw animals in from long distances we assume this random movement of individuals a short distance will have a negligible effect on our results.

We used circuit theory to estimate pairwise resistance distances between all pairs of individuals using the software CIRCUITSCAPE (McRae 2006; McRae *et al.* 2008). In circuit theory connectivity across the landscape is estimated using electrical theory where individuals act as nodes connected by resistors which conduct current. In a landscape resistance model the resistor is defined as a habitat type that hypothetically impedes the movement of an organism (pixel values > 1). Circuit theory is an appealing model for ecology as it considers all possible paths of connectivity across the landscape, accounts for effects of path size (wider paths will have greater conductance) and has a direct relationship with random walks (McRae *et al.* 2008). CIRCUITSCAPE analyses were conducted in a pairwise mode, with individual locations set as focal nodes, and each pixel connected to its 8 neighboring pixels.

Because the relationship between a landscape feature and its corresponding resistance surface is unknown we tested landscape features as both continuous and categorical variables over a wide range of maximum resistance values ranging from 2 to 100 (Table 4-1). We also established a null model representing Euclidean distance between individuals by running a CIRCUITSCAPE model with a homogeneous resistance surface, where all pixels were given a resistance value of 1. This null model has been established as the appropriate surrogate for geographic distance between individuals for circuit theory analyses instead of pure Euclidean distance as it accounts for the edge effect, where pairwise resistance distance increases toward the edge of the grid (Koen *et al.* 2010, Amos *et al.* 2012). All references to Euclidean distance in the remainder of this document refer to this null model.

Optimizing resistance values

We calculated genetic distance between each pair of individuals as the proportion of shared alleles (Bowcock *et al.* 1994) using the software package Microsatellite Analyzer (Dieringer & Schlotterer 2003). We then assessed the optimal resistance value for each variable using partial mantel tests which measure the association between two dissimilarity matrices while controlling for the effect of third matrix (Mantel 1967; Smouse *et al.* 1986). There has been criticism of the

use of partial mantel tests in landscape genetics (Legendre & Fortin 2010; Guillot & Rousset 2013), but this criticism has primarily focused on bias in the statistical significance of the tests. We used these tests to evaluate relative strength of the relationship between each variable and genetic distance while controlling for Euclidean distance. For each variable we tested a matrix of pairwise genetic distance against pairwise resistance distances, partialling out Euclidean distance, and repeated this test for the range of maximum resistance values (max resistance = 2 to 100). Mantel and partial mantel tests were conducted using the *Ecodist* package in R (Goslee & Urban 2007). To investigate the potential differences in gene flow between males and females we conducted resistance value optimization for males and females both separately and combined. Optimum resistance was defined by the asymptote of the curve (rate of change <5%) of the partial mantel r against the maximum resistance value, and we visualized this relationship using the R package *ggplot2* (Wickham 2009). When the partial mantel values for males and females substantially differed we based the optimum resistance value on the sex that had the strongest relationship with genetic distance.

Model selection and evaluation

We used the optimized resistance values to fit linear models of landscape resistance to genetic distance, with statistical significance assessed using multiple regression of distance matrices (MRDM) (Manly 1986; Legendre *et al.* 1994). MRDM is a multiple regression of a response distance matrix against two or more environmental variables in which significance testing is performed by random permutation of the response distance matrix (Lichstein 2006). In landscape genetics MRDM models the response variable is the genetic distance between individuals and the environmental variables are the resistance distance between individuals for each landscape feature (i.e. $DIST_{GENETIC} \sim DIST_{RESISTANCE1} + DIST_{RESISTANCE2} + \ldots + DIST_{RESISTANCEn}$). MRDM is a method that has been found to have high power and low type-1 error for landscape genetic analyses (Balkenhol *et al.* 2009).

To test our hypothesis that landscape features may influence males and females separately we fit an identical suite of MRDM models to each of three groups: all individuals, males only, and females only. To test our hypothesis that landscape features important to gene flow may vary in different portions of the study area we partitioned the study area based on the subpopulation structure identified in Tucker *et al.* (In Review) into two geographic regions consisting of each of the two adjacent subpopulation pairs (North-Central and Central-South) for comparison to an analysis using the entire study area (Figure 1). We chose to subdivide in this manner because it excludes the largest pairwise distances from analyses (North-South) which were on average more than twice the maximum reported dispersal distance for fisher of ~ 107 km (York 1996), making it unlikely that an individual directly disperses between the North and South groups. Including these long distance pairs may overestimate the importance of long distance landscape connectivity over landscape connectivity within the actual dispersal range of the species (Parks *et al.* 2013). The combination of partitioning both by sex and by region

resulted in 9 separate analyses (all individuals, males, females X all subpopulations, North-Central region, Central-South region). We then used a model selection approach to identify the landscape resistance model best supported by the genetic data for each of these 9 analyses.

Prior to creating a candidate model set, we assessed variable multicollinearity by calculating Pearson's correlation between all pairs of variables and variance inflation factors (VIF) for a global linear model containing all variables. During this initial assessment we found evidence for very high collinearity between many of the landscape variables and between each landscape variable and Euclidean distance (Pearson's correlations>0.95, VIFs>100). We discerned that this collinearity was generated in part because each landscape resistance distance matrix from CIRCUITSCAPE includes Euclidean distance such that all variables will be collinear to some degree due solely to this repeated inclusion of Euclidean distance. To address this problem we subtracted Euclidean distance from the resistance distances for each variable prior to fitting MRDM models. After subtracting out Euclidean distance we again calculated Pearson's correlations between all variable pairs. Any pair of variables with a correlation>0.70 were excluded from being in the same candidate model (Table 4-S1).

We created a set of 23 candidate models (4 univariate, 19 multivariate) from our 8 landscape variables to test our hypotheses of landscape resistance and fisher gene flow shaped by 3 overarching hypotheses: 1) fisher gene flow is facilitated by mid-elevation dense forest habitat strongly associated with fisher occupancy (Spencer *et al*. 2011), 2) fisher gene flow is facilitated by dense forest across a broad elevation range including high elevation dense forest, and 3) fisher gene flow is impeded by open areas such as large water bodies, roads, and burned areas (Table 2-S2). We also included the null model representing Euclidean distance for comparison to our resistance models. Because the optimum resistance value for each variable differed from 2 to 100, we standardized all variables using a z-transformation to facilitate comparison of model parameter estimates. Subsequently, we fit MRDM models of pairwise genetic distance and candidate models using 10,000 permutations for significance tests. Models were again assessed for multicollinearity and any model which had a variable with a VIF >5 was dropped from the candidate model set.

Models were ranked using second order AICc values. We then examined the top model set (ΔAIC<7) for uninformative parameters which occur in models that differ from the top model by only one parameter but do not improve the maximized log-likelihood. Given that AIC values are calculated as AIC=2k-2ln(L), where K is the number of parameters and L is the maximized value of the likelihood function, such models will only differ from the top model by 2 AIC units due to the addition a parameter even though they do not add any explanatory power. Therefore, we excluded models from that were similar to the top model except for an additional uninformative parameter that did not improve the AIC score by at least 2 (Burnham & Anderson 2002; Arnold 2010). We also evaluated the variables within the top model using the 85%

confidence intervals (CI) of the parameters as CIs overlapping zero are indicative of a variable that is not ecologically important (Arnold 2010). We reported 85% CIs as they have been shown to be more compatible with AIC model selection than the more traditional 90% or 95% CIs (Arnold 2010). All steps of model fitting and model selection were conducted in R (Team 2012; R Development Core Team 2012) using the following packages: *GenABEL* for ztransformations (Aulchenko *et al.* 2007), *car* for VIF calculation (Fox & Weisberg 2011), *ecodist* for MRDM (Goslee & Urban 2007), and *MuMIn* for model selection and model averaging (Barton 2013).

Finally, we further evaluated the relationship for the top models by using the model equation derived from MRDM to create a single resistance surface from the component parameter estimates using the Spatial Analyst extension of ArcGIS 10 (ESRI 2011). We used the resulting resistance surfaces as input in CIRCUITSCAPE to calculate landscape resistance distances between all pairs of individuals. We used partial Mantel tests to assess the correlation between the resulting matrices of pairwise resistance distances and genetic distances.

Results

As detailed in Chapters 2 and 3 (Tucker *et al.* 2012; Tucker *et al.* In Review), we genotyped 127 individuals at 10 microsatellite loci including 72 males, 48 females, and 7 individuals for which we were not able to conclusively assign sex. Sample sizes by subpopulations identified in Chapter 3 (Tucker *et al*. In Review) were as follows: North n= 44 (16 females, 24 males, 4 unknown sex), Central n=32 (12 females, 19 males, 1 unknown sex), and South n=51 (20 females, 29 males, and 2 unknown sex).

Sex-biased dispersal

Tests for sex-biased dispersal yielded mixed results but suggested that males disperse more than females. We found F_{ST} among the three subpopulations for males to be significantly lower than the F_{ST} values for females (Table 4-2). Within subpopulations pairs (North/Central or Central/South), the F_{ST} test was highly significant in the North/Central pair (F_{ST} (fem) = 0.17: F_{ST} (male) = 0.06, p = 0.004) and non-significant in the Central/South pair (F_{ST} (fem) = 0.07: F_{ST} (male) = 0.05, p = 0.18). We also found a tendency for an excess of homozygotes within subpopulations for males ($F_{IS} > 0$), but not for females, as is expected if males disperse more than females (F_{IS} North: Male = 0.053, Female = 0.006; F_{IS} Central: Male = 0.043, Female = -0.005; F_{IS} South: Male = 0.043, Female = 0.033). However, none of the F_{IS} values were statistically significant from zero with all p values > 0.05.

All assignment index tests for differences between the mean and variance AI for males versus females were non-significant (Table 4-2). The values of the mean and variance in AI were consistent with male-biased dispersal when testing between just the North and Central subpopulations (mean AI > 0 and low variance = philopatric sex; mean AI<0, higher variance=

dispersing sex) but had had values in the opposite directions in the Central/South subpopulations and for all 3 subpopulations combined.

Assignment in GENELAND found K=3 for both males and females with similar boundaries between each of the three subpopulations. However, females assigned more strongly in these subpopulations than males (mean probability of assignment: female=0.98 (sd=0.04), male=0.87 (sd=0.09), with a higher F_{ST} value between subpopulations for females than for males (Table 4-3).

Optimizing resistance surfaces

We found that partial mantel correlations of genetic distance and resistance distance were considerably different for males and females for the majority of variables (Figure 4-2). When analyzing the sexes together partial Mantel correlations generally were an intermediate value between the values for each sex, with the partial Myantel *r* associating more closely with the partial Mantel *r* for males that had the larger sample size of the two sexes. Optimum resistance values varied considerably for different variables ranging from 2 (openings, fire) to 100 (dense forest) (Table1).

Model selection and evaluation

Model selection varied substantially between sexes and between geographic regions, in terms of the variables included in the top models, the magnitude and direction of parameter estimates, and model fit (Table 4-4). Across all subpopulations, the top models for females showed positive relationships between genetic distance and resistance distances for water (β_{water}=0.21), roads (β_{roads}=0.16), and dense forest ($\beta_{dforest}$=0.13). Both water and roads were defined as features resistant to gene flow therefore, the positive relationship observed between genetic distance and these features indicates that for females both large water bodies and roads impede gene flow. Conversely, the landscape feature of dense forest was defined as conductive to gene flow and as such the positive association between dense forest and genetic distance indicates that dense forest facilitates gene flow.

The top model for males included positive relationships with Sierra Mixed Conifer (SMC) forest (β_{SMC}=0.30), and slope (β_{slope} = 0.08) and a negative relationship with water (β_{water}= -0.11). Sierra Mixed Conifer forest was defined as conductive to gene flow, and slope as resistant to gene flow so the positive relationship with these variables indicated that for males Sierra Mixed Conifer forest facilitate gene flow while steep slopes impede it. Water was the only variable in common in the top models for males and females, but the direction of the relationship with genetic distance was opposite in the two sexes such that for males water bodies do not impede gene flow.

When both sexes were combined the top model for all subpopulations was similar to the top model for males. As previously noted this is likely due to the larger sample size for males in our dataset. For individuals across all subpopulations a single top model was clearly selected (model weight=1.00) but this model had a poorer model fit and the resulting resistance surface had a much lower partial Mantel correlation with genetic distance than the resistance surfaces for either males or females. ($r_{pm\ (all)}$=0.05, $r_{pm\ (females)}$=0.24, $r_{pm\ (males)}$=0.14).

Partitioning individuals geographically revealed that not only did top models vary by sex, but also between regions (Table 4-4). The top model for females in the North-Central subpopulations showed that gene flow in this region was most strongly influenced by water (β_{water} = 0.34) and the variable for roads was not included in the top model for this region. The top model in the Central-South showed roads having a strong association with genetic distance (β_{roads}=0.31) and water having a smaller effect than in the North-Central region (β_{water}=0.11). Additionally, the variables of slope (β_{slope}=0.15), and fire (β_{fire}=0.10) were found in the top model for males in the North-Central region but not in the Central-South.

Females had better model fit than males alone or both sexes combined across all geographic groups, with females in the North-Central region having the best model fit and highest partial Mantel correlation with the resulting resistance surface of any sex or region (R^2 = 0.25, r_{pm} = 0.33). When sexes were combined the top models in both regions were a mix of variables from the male and female models and had relatively poor model fit compared to females alone. Notably, the top model for all individuals in the North-Central region included a negative parameter estimate for water despite the strong positive association found for females in this region. Top models for all sexes and regions were statistically significant at $p < 0.01$.

Discussion

Landscape genetics research has evaluated a variety of factors that can affect the identification of landscape features responsible for gene flow including evaluation of spatial scale and thematic resolution (Cushman & Landguth 2010), timescale (Landguth *et al.* 2010b), and migration rate (Landguth *et al.* 2010b). While other genetic studies have examined at the strength of correlation with landscape features for males and females independently (Coulon *et al.* 2005)**,** to our knowledge no study has explicitly considered in an individually based landscape genetics framework how landscape features that influence dispersal may differ by sex. The study of sex-biased dispersal is complex and draws inferences from many diverse fields including evolutionary theory, animal behavior, and demography (Lawson, Handley & Perrin 2007) and this study demonstrates that landscape genetics provides another avenue by which we can add to our understanding of this important aspect of a species life history.

Our results show that both sex-biased dispersal and spatial landscape heterogeneity can considerably affect determination of the landscape features associated with gene flow. By

conducting resistance modeling for each sex independently we found that different landscape features influence gene flow for each sex. Our results also show that for a species characterized by sex-biased dispersal, conducting analyses with sexes combined can produce misleading results in which important landscape variables are either not detected or are detected as having the incorrect relationship with genetic distance resulting in a model that has poor fit with the genetic data. This finding has considerable potential implications for research and conservation in that failing to account for sex-biased dispersal in landscape genetics analyses may result in omitting or mis-identifying the landscape features important for genetic connectivity.

We also found that when our analysis was conducted with both sexes combined that 1) the sex with the larger representation in the sample dominated the results such that landscape features influential for the sex with a smaller sample size are not detected, and 2) if a landscape feature had an opposing relationship between the sexes (one sex has a positive relationship and the other a negative relationship) then the variable is either not included in the top model, or if it is included, then the parameter estimate is often an intermediate value between that of the two sexes weighted toward the sex with a larger sample size. We acknowledge that the sample sizes used in this study are small, which has been show to decrease the power to correctly identify landscape relationships in some types of landscape genetics analyses (Landguth *et al.* 2012). However, this issue has never been directly addressed using a MRDM and model selection approach. The sample sizes used in this study are similar to the within population sample sizes of other landscape genetic analyses using MRDM that detected strong relationships between genetic distance and landscape features (Garroway *et al.* 2011; Trumbo *et al.* 2013).

We also found that different landscape features in different geographic regions affect gene flow. Similar to our results for sex-biased dispersal, landscape variables detected as important when analyzing the entire study area differed from those detected in each geographic region. These differences were most pronounced when analyzing data with the sexes combined. When males and females were analyzed separately the top models for the entire study area compared with regional subsets were relatively congruous in terms of the variables included in the model. However, parameter estimates varied considerably by region, and as a result the parameter estimates for the overall population tended to be an average of the two regional values. This resulted lower model fit for the overall population compared to the regional models. Our results concur with other studies that have found spatial heterogeneity in landscape genetic relationships, and we echo the warning that it may not be appropriate to apply landscape genetic findings from a specific area to other populations within a species (Short Bull *et al.* 2011; Trumbo *et al.* 2013).

Sex-biased dispersal

There are some inherent difficulties in detecting sex-biased dispersal using bi-parentally inherited markers. In a population with non-overlapping generations, these markers can detect

the signal of sex-biased dispersal only after dispersal but before production of a new generation of offspring. This is because all offspring inherit half their genes each from their mother and father. Consequently, if sex-bias dispersal ceases the genetic signal dissipates after one generation (Prugnolle & de Meeus 2002; Goudet *et al.* 2002). The presence of overlapping generations, which is more realistic of wild populations, will extend the lifespan of the genetic signal of sex-biased dispersal to some extent as mothers and daughters cluster geographically while male offspring disperse farther from their natal area, but the sex-biased signal still dissipates over time. The statistical power to detect a sex bias using nuclear DNA also depends on the rate of dispersal such that if there are many dispersers the genotypes of both sexes may become homogenous, while if dispersers are very rare than their genotypes may not have enough an effect on overall genetic variation to be detectable. Thus, the power to detect sex-biased dispersal may be limited in certain populations (Goudet *et al.* 2002). Comparisons between markers with different inheritance patterns (mtDNA and nuclear DNA) can provide valuable insight into sex-biased dispersal over longer time frames but these methods were not useful in our study as the southern Sierra Nevada fisher population is fixed for a single mtDNA haplotype, even based on analyses of the entire mitochondrial genome (Drew *et al.* 2003; Knaus *et al.* 2011).

Our tests for sex-biased dispersal yielded mixed results, but suggest that males disperse more than females. We found a significant difference in the F_{ST} between subpopulations for males and females, $F_{IS} > 0$ for males but not for females, but no significant difference in the mean or variance of AI. Such mixed results have been reported in other studies of species that otherwise have strong evidence for sex-biased dispersal (Helfer *et al.* 2012). Simulation studies by Goudet *et al.* (2002) found for species with moderate to high dispersal rates (>10%) the F_{ST} test is the most powerful and least sensitive to changes in sampling scheme and magnitude of sex-bias. Thus, our finding of a significant result only in the F_{ST} test is indicative of sex-biased dispersal with moderate to high dispersal rates for males. Notably while the F_{ST} test was significant for the entire population, regionally it was only significant for the North-Central groups and not for the Central-South. This is likely being driven by higher overall gene flow between the Central and South groups as evidenced by the much lower F_{ST} value for both males and females compared with the North–Central F_{ST} values. When there are high rates of dispersal, populations are less differentiated and dispersers are more difficult to detect resulting in a reduction in statistical power of the tests (Goudet *et al.* 2002). However, while the difference was not statistically significant the F_{ST} of females in the Central-South is higher than that of males, and is consistent with the expectation for F_{ST} and female philopatry. The results of our population assignment analyses in GENELAND are also consistent with sex-biased dispersal, with females assigning more strongly than males to all three subpopulations.

The tests for sex biased dispersal discussed above (Goudet *et al.* 2002) are population based and therefore, only reflect dispersal between subpopulations. However, it is important to

note that the MRDM resistance modeling approach we employed is individually based. Therefore, the results of MRDM models fitting pairwise genetic distance to resistance distances between all individuals, reflects dispersal movements both within and between subpopulations. Using a population based approach one individual dispersing between subpopulations may only have a small effect on F_{ST}, but this one disperser will affect the pairwise values for genetic and resistance distances to all other individuals both within and between subpopulations. For example if only 8 of 72 of male fisher in the southern Sierra Nevada disperse between subpopulations (~11% male dispersal), this will affect 284 pairwise distances between individuals. This distinction has important implications in the determination of how landscape features influence gene flow. Population based analyses will reflect the influence of landscape features between subpopulations, whereas individual based analyses reflect the influence of features across the entire landscape, both within and between subpopulations.

Landscape features influencing gene flow

Our results indicate that analyses conducted for males and females independently are more biologically relevant to a discussion of the landscape genetics of fisher in the southern Sierra Nevada. We reached this conclusions based on: 1) the observed differences between sexes in the partial mantel correlations using individual variables during optimization of resistances surfaces, 2) the considerable differences between sexes in the variables and parameters estimates of the top models, and 3) the large improvement in model fit when analyzing data by sex as compared to all individuals combined.

For females the variables of water and dense forest were consistently detected in all three geographic groups, with roads present in the top model of two of the three geographic groups. The inclusion of dense forest (>60% canopy cover) in all top models is consistent with home range characteristics for fishers in the Sierra Nevada (Zielinski *et al.* 2004; Purcell *et al.* 2009) and throughout their range (Schwartz *et al.* Accepted; Aubry *et al.* In Review). The strength of the relationship varied regionally with water showing the strongest fit to genetic data between individuals in the North-Central group. This is likely attributable to the Kings River Canyon, which has long been hypothesized to limit fisher gene flow (Wisely *et al.* 2004; Jordan 2007). Within the study area the Kings River canyon reaches a maximum depth of 8,200 feet (2,500 meters) from the Kings River to the summit of the adjacent mountains, so it is unsurprising that such a major landscape feature impedes gene flow. The strong relationship with water and genetic distance in this region may also be attributable to the San Joaquin River which has been hydrologically altered by a series of dams, including the large Mammoth Pool Dam and reservoir which bisects the North subpopulation.

In the Central-South region we found roads had the strongest relationship to genetic distance, with water still present in the top model but not statistically significant. This strong relationship between genetic distance and roads in this region was unexpected as the density of

roads and intensity of road use seems comparable across regions. The most heavily used roads within the Central-South road system are located within and adjacent to Sequoia and Kings Canyon National Parks in the Central group, while in the South group highway 190 bisects fisher habitat on the Sequoia National Forest. However, all of these roads are primarily 2 lane mountain roads running through otherwise contiguous forested habitat, and we did not expect to find such a strong effect on gene flow. We are unable to discern from this analysis if all or just a portion of these roads is responsible for the observed relationship.

We found an entirely different suite of variables in the top models of males. Water was detected in all three top models but had a consistently negative relationship with genetic distance indicating that water bodies do not act as a feature resistant to gene flow but rather facilitate gene flow for males. Considering the aforementioned magnitude of the Kings River Canyon it is surprising that this feature does not appear to create any impediment to dispersal in males. However, resistance due to steep slopes and burned areas were included in the model for the North Central group. In this region steep slopes and fire scars as are generally associated with river canyons and so may be an alternate reflection of the effect of these features on gene flow.

The variable for Sierra Mixed Conifer forest was also present in all male top models instead of dense forest variable found important in females. The difference between males and females for this single WHR class is a particularly intriguing difference between the sexes. The Sierra Mixed Conifer variable encompasses a higher elevational range than the tree classes that comprise the dense forest variable (maximum elevation Sierra Mixed Confier = 2602m (8536 ft), maximum elevation dense forest = 2223 m (7293 ft). The Sierra mixed conifer variable also covers a much larger geographic area covering an additional 171,849 pixels in the landscape over the dense forest layer. We speculate that this indicates that females gene flow is characterized by dispersal among high quality habitat in the core elevation range for fisher occupancy in the Sierra (~1400 - 2300 m elevation) (Spencer *et al.* 2011), while males disperse more widely and therefore, strongly associate with a more widespread landcover type. However, it is important to emphasize that model fit for males was markedly lower for all geographic groups than for females. This indicates that landscape features overall have much less influence on gene flow for males compared to females. The poor model fit and low partial mantel correlations of the final resistance surfaces implies that for males these landscape create only minor resistance to gene flow.

Our results have important implications specifically for fisher conservation and management within the Sierra Nevada and broadly for landscape genetic analyses for any population characterized by sex-biased dispersal or spatial heterogeneity in landscape features. For the Sierra Nevada fisher population our results have repercussions for assessing the potential future expansion of fishers beyond the current geographic extent in the southern Sierra Nevada. This population is currently limited south of the Merced River in Yosemite National Park.

However, habitat models for this population have indicated that there is suitable but unoccupied habitat north of the Merced River (Davis *et al.* 2007; Spencer *et al.* 2011) and incidental sightings of fishers north of the Merced River indicate that this feature is not a complete barrier to movement (Chow 2009). Our findings indicate that female dispersal may limit population expansion north of its current extent. The northern boundary of the current population aligns with two features shown to strongly impede female fisher flow; the Merced River canyon and the heavily traveled roads associated with Yosemite National Park which averages 3.7 million visitors per year (National Park Service 2012). If expanding the population north of the Merced River is deemed critical to maintain the long-term viability of this population, as is currently being discussed, then it may be necessary to facilitate this by translocating females across the Merced River to establish a population and maintain genetic connectivity with the rest of the fisher population or by mitigating the highway with crossing structures.

Conclusions

This study demonstrates that for species with sex-biased dispersal the influence of landscape features on gene flow may be very different for males and females. Because of this difference conducting landscape genetic analysis separately for each sex allows for the identification of landscape genetics relationships not discernible when the sexes are analyzed together. This result has potential implications for the conservation of genetic connectivity and long-term population persistence for any species that exhibits sex-biased dispersal. Our results indicate that management actions with the goal of conserving or enhancing population connectivity need to consider that males and females may each have a different suite of habitat features important for connectivity. For species with male-biased dispersal, population expansion is likely mediated by the female's ability to disperse to new habitat areas. Consequently, identifying and conserving landscape features important for female dispersal may be more important than conservation of landscapes for males that disperse more widely.

Our results suggest that careful consideration of the potential for sex-biased dispersal as well as spatial heterogeneity in a landscape must be undertaken prior to conducting landscape genetic analyses as these factors can strongly influence results. In fact failing to account for sex-biased dispersal in landscape genetic analyses can confound results and obscure the relationship between landscape features and gene flow. For populations in which such factors are either known or suspected we recommend conducting analyses separately for males and females and spatially replicating analyses over multiple geographic areas to assess how these factors affect results. While this study has empirically demonstrated how sex-biased dispersal can affect landscape genetic results more work is needed to identify under what specific conditions sex-biased dispersal will influences these type of analyses (i.e. dispersal rate, magnitude of sex-bias). Given the complexities of assessing dispersal with field data we feel that genetic simulations are likely the best tool to begin to better define this issue.

Table 4-1. Description of landscape variables hypothesized to influence fisher gene flow.

Feature	Description	Obtained at:	Continuous	Categorical	Optimum resistance
Water	major lakes (>35 ha) and rivers (including all forks) are resistant to gene flow	atlas.co.gov/download.html#/casil/inlandWaters	density (per 1km moving window)	water / non-water	categorical resistance=25
Roads	primary and secondary roads are resistant to gene flow	http://geo.data.gov/geoportal/catalog/main/home.page	density (per 1km moving window)	road/non-road	categorical resistance =5
Slope	steep slopes are resistant to gene flow	calculated from elevation using ArcGIS 10, Spatial Analyst extension	raw slope values (percent)	slopes>threshold = resistance Test 4 thresholds: 50, 60, 70, 80% slope	categorical: 70% slope resistance=25
Elevation	latitude adjusted elevation: mid elevations facilitate gene flow while low and high elevations are resistant to gene flow.	Conservation Biology Institute http://consbio.org/	inverted Gaussian function of raw values (low resistance at mid elevations)	4 categories: 500-999 ft = high 1000-2000 ft =moderate 2000-3000 ft =low >8000 ft =low 3000-8000 ft= 1	categorical 500-999 ft = 20 1000-2000 ft =15 2000-3000 ft =10 >8000 ft =10 3000-8000 ft= 1
Dense forest	dense forest facilitates gene flow and non-forested areas are resistant to gene flow. CAWHR Density 'D'=60% canopy cover; WHR Size=4&5, WHR Type=Doug Fir, White Fir, Ponderosa Pine, Jeffery Pine, Eastside Pine, Montane Hardwood Conifer	selected from existing vegetation (eveg) layer (Conservation Biology Institute)	percent of forest within a 500 x 500m neighborhood	dense forest/not dense forest	categorical resistance=100
Sierra Mixed Conifer	same as for dense forest with the addition of the Sierra Mixed Conifer habitat type.	Conservation Biology Institute	% forest type within a 500 x 500m neighborhood	dense forest/not dense forest	categorical resistance=10
Fire	areas recently burned in moderate to high severity fires (1984-2005) are resistant to gene flow	U.S. Forest Service: www.fs.usda.gov/detail/r5/landmanagement/gis	N/A	burned/non-burned	categorical resistance=2
Openings	proportion of 1km neighborhood consisting of non-forested cells (all WHR shrub classes, grassland classes, sagebrush, and barren)	Conservation Biology Institute existing vegetation (eveg) layer	percent of a 1 km radius neighborhood	>50% of neighborhood open= resistance <50% open= no resistance	categorical resistance=2

Table 4-2. Tests for sex-biased dispersal between males and females for F_{ST} and the mean and variance of the Assignment Index (AI) with individuals are assigned to one of three genetic subpopulations.

	F_{ST}	Mean AI	Var AI
North/Central/South			
Female	0.133	-0.036	5.881
Male	0.083	0.023	4.925
p value	0.032*	0.553	0.650
North/Central			
Female	0.170	0.217	3.944
Male	0.055	-0.136	4.856
p value	0.004*	0.275	0.592
Central/South			
Female	0.072	-0.181	6.838
Male	0.048	0.117	4.104
p value	0.175	0.689	0.866

Table 4-3. F_{ST} values calculated based on subpopulation assignment in GENELAND conducted separately for males and females. F_{ST} values for females are above the diagonal and males below the diagonal. All F_{ST} values in this table are significant at $p<0.01$.

	North	Central	South
North	0	0.173	0.155
Central	0.059	0	0.069
South	0.132	0.051	0

Table 4-4. Variables and parameter (β) estimates for the top model for each sex and geographic group. w_i = model weight, R^2=fit of MRDM model, r_{pm}=partial mantel of overall resistance surface.

All Populations

All (n=127)				Female (n=48)				Male (n=72)			
	β	85% CI	p		β	85% CI	p		β	85% CI	p
water	-0.07	(-0.09, -0.06)	0.09	water	0.21	(0.15,0.28)	0.02	water	-0.11	(-0.14, -0.08)	0.10
SierraMC	0.27	(0.25, 0.28)	<0.01	roads	0.16	(0.12, 0.20)	0.12	SierraMC	0.30	(0.27, 0.32)	<0.01
slope	0.09	(0.07, 0.10)	0.07	dense forest	0.13	(0.07, 0.19)	0.16	slope	0.08	(0.05, 0.11)	0.22
w_i=1.00	R^2=0.10	r_{pm}=0.05		w_i=0.97	R^2=0.15	r_{pm}=0.24		w_i=1.00	R^2=0.11	r_{pm}=0.14	

North & Central Subpopulations

All (n=76)				Female (n=28)				Male (n=43)			
	β	85% CI	p		β	85% CI	p		β	85% CI	p
water	-0.09	(-0.12, -0.06)	0.19	water	0.34	(0.24, 0.43)	<0.01	water	-0.16	(-0.22, -0.10)	0.14
dense forest	0.21	(0.18, 0.24)	**0.13**	dense forest	0.20	(0.11, 0.29)	0.114	SierraMC	0.20	(0.14, 0.26)	0.05
slope	0.11	(0.08, 0.14)	<0.01					slope	0.15	(0.10, 0.20)	0.07
fire	0.13	(0.11, 0.16)	<0.01					fire	0.10	(0.05, 0.15)	0.07
w_i=0.99	R^2=0.10	r_{pm}=0.11		w_i=0.94	R^2=0.25	r_{pm}=0.33		w_i=0.59	R^2=0.06	r_{pm}=0.16	

Central & South Subpopulations

All (n=83)				Female (n=32)				Male (n=48)			
	β	85% CI	p		β	85% CI	p		β	85% CI	p
roads	0.19	(0.16, 0.21)	**<0.01**	water	0.11	(0.03, 0.19)	0.31	water	-0.10	(-0.14, -0.05)	0.271
SierraMC	0.19	(0.17, 0.21)	<0.01	roads	0.31	(0.24, 0.37)	<0.01	SierraMC	0.22	(0.18, 0.27)	<0.01
				dense forest	0.16	(0.09, 0.24)	0.131				
w_i=0.92	R^2=0.05	r_{pm}=0.16		w_i=0.68	R^2=0.148	r_{pm}=0.33		w_i=0.92	R^2=0.05	r_{pm}=0.08	

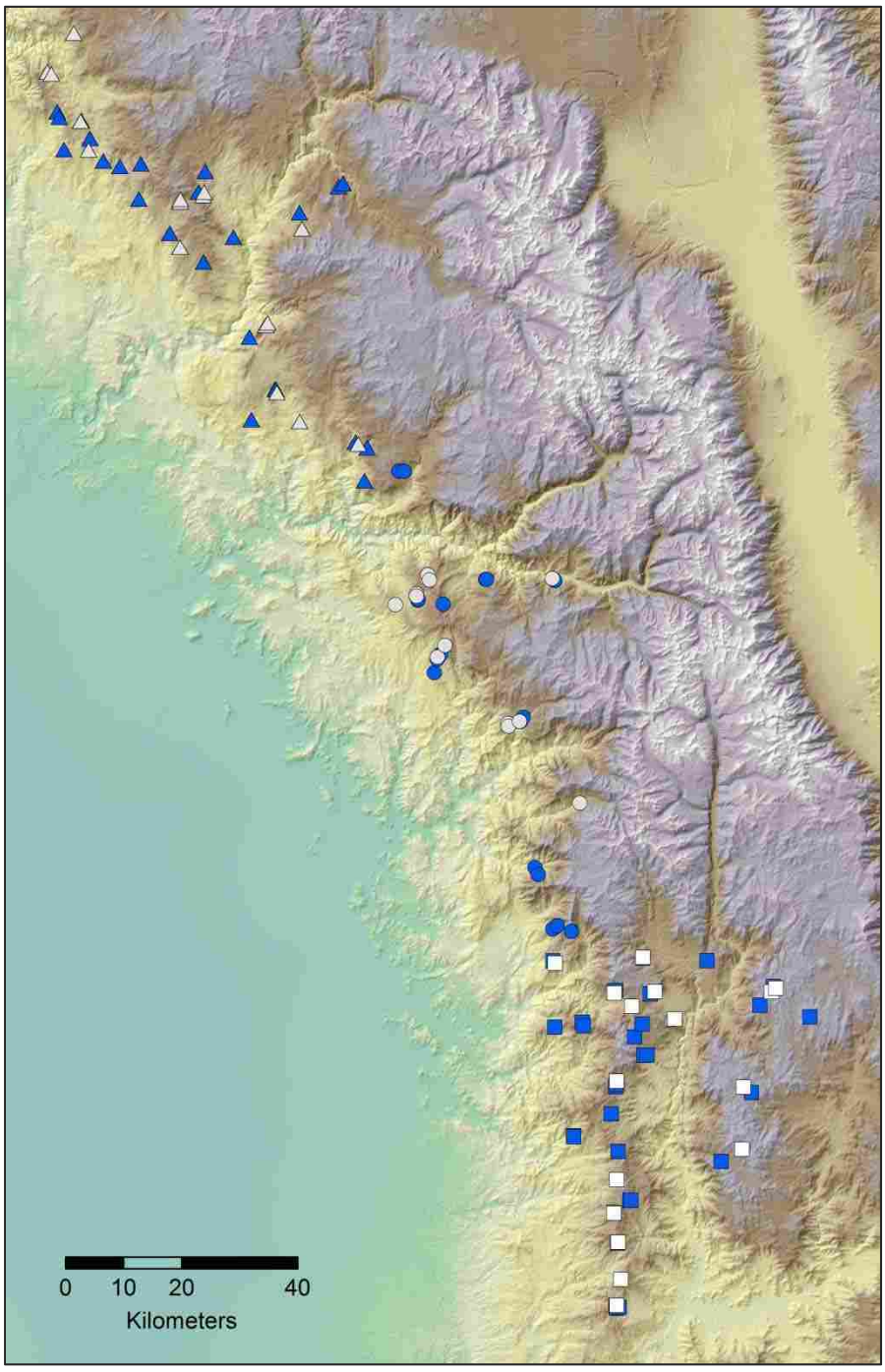

Figure 4-1. Locations of individual fishers by sex (male=blue, female=white) and their assignment to three genetic subpopulations (North=triangle, Central=circle, South=square)

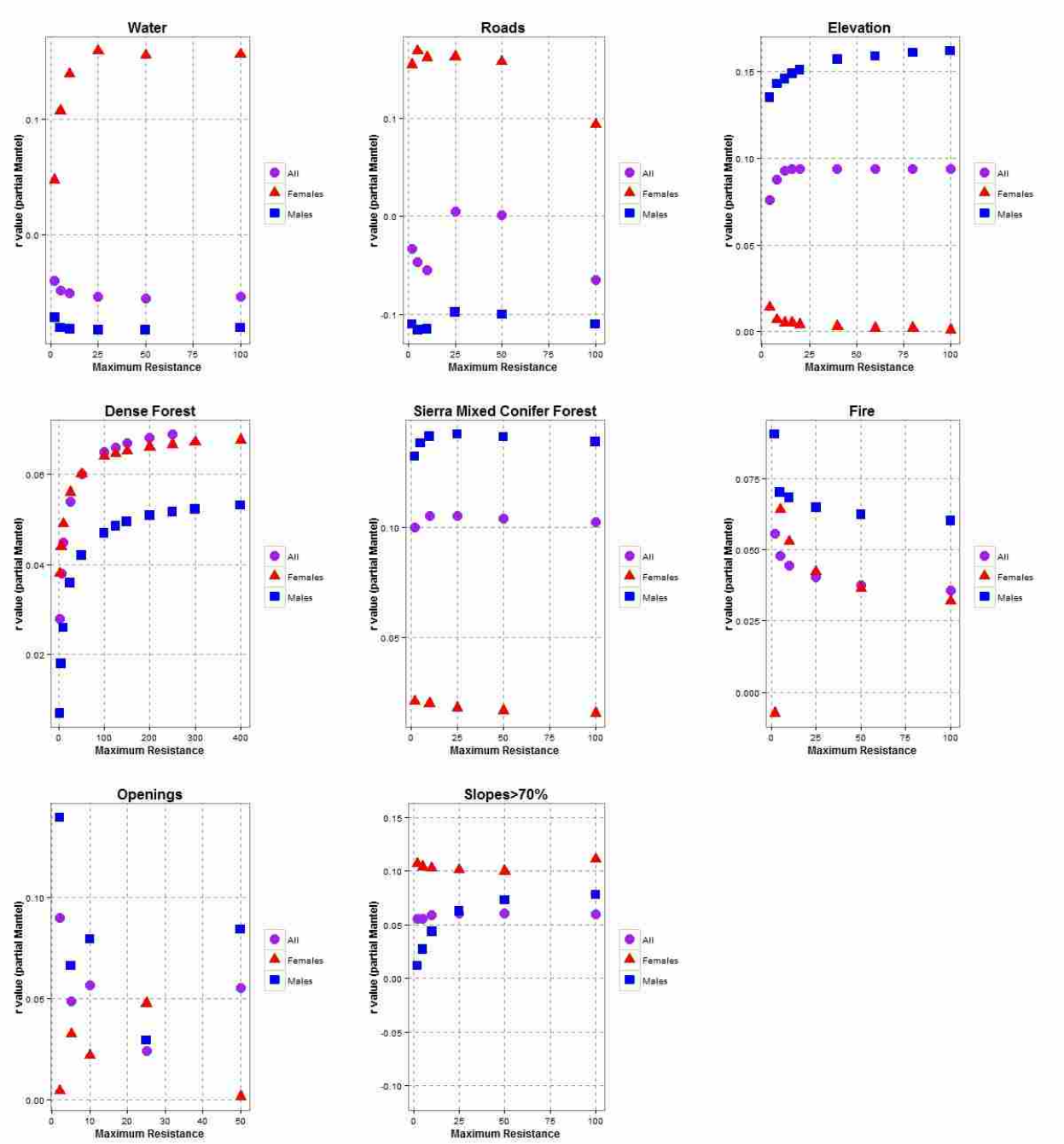

Figure 4-2. Plots of the partial Mantel correlation against a range of maximum resistance values (2-100) for all individuals and for each sex independently.

Supplemental Tables

Table 4-S1: Pearson's correlations between resistance variables. SMC = Sierra Mixed Conifer habitat type.

	Water	Roads	Slope	Elevation	SMC Forest	Dense Forest	Fire	Openings
Water	1							
Roads	-0.01	1						
Slope	0.15	-0.01	1					
Elevation	0.07	0.01	0.27	1				
SMC Forest	0.20	0.00	0.40	**0.77**	1			
Dense Forest	0.13	-0.01	0.47	0.65	**0.90**	1		
Fire	-0.03	-0.03	0.01	0.37	0.37	0.27	1	
Openings	0.11	0.02	0.41	0.64	**0.87**	**0.87**	0.31	1

Table 4-S2. Candidate model set representing hypotheses of the influence of landscape features on fisher gene flow. The dependent variable for all models is the genetic distance between individuals. All models were fit using multiple regression of distance matrices (MRDM).

Candidate Models
Null
$GENETIC_{DIST}$ ~EUCLIDEAN DISTANCE
Barrier
$GENETIC_{DIST}$ ~WATER
$GENETIC_{DIST}$ ~ROADS
$GENETIC_{DIST}$ ~WATER + ROADS
$GENETIC_{DIST}$ ~WATER + ROADS + SLOPE
$GENETIC_{DIST}$ ~OPENINGS + SLOPE
$GENETIC_{DIST}$ ~OPENINGS + FIRE
$GENETIC_{DIST}$ ~OPENINGS + FIRE + WATER
Dense Forest (DFOR)
$GENETIC_{DIST}$ ~DFOR
$GENETIC_{DIST}$ ~DFOR + WATER
$GENETIC_{DIST}$ ~DFOR + ROADS
$GENETIC_{DIST}$ ~DFOR + SLOPE
Sierra Mixed Conifer (SMC)
$GENETIC_{DIST}$ ~SMC
$GENETIC_{DIST}$ ~SMC + SLOPE
$GENETIC_{DIST}$ ~SMC + FIRE
$GENETIC_{DIST}$ ~SMC + WATER
$GENETIC_{DIST}$ ~SMC + ROADS
Global
$GENETIC_{DIST}$ ~DFOR + WATER + ROADS
$GENETIC_{DIST}$ ~DFOR + WATER + ROADS + SLOPE
$GENETIC_{DIST}$ ~DFOR + WATER + SLOPE + ELEVATION
$GENETIC_{DIST}$ ~DFOR + WATER + SLOPE + FIRE
$GENETIC_{DIST}$ ~DFOR + ROADS + ELEVATION
$GENETIC_{DIST}$ ~SMC + WATER + SLOPE
$GENETIC_{DIST}$ ~SMC + WATER + SLOPE + FIRE

CHAPTER 5: Using spatially explicit power analyses to determine factors influencing the power to detect trend for the southern Sierra Nevada fisher population

Abstract

Determining whether a population is increasing, decreasing, or stable over time (population trend) is a fundamental component of species listing decisions and recovery plans. We used a spatially explicit simulation approach that accounts for natural history, habitat use, and sampling scheme to investigate the factors that influence statistical power to detect population trends in occupancy. We simulated a declining population of fisher (*Pekania pennanti*) and recreated the sampling scheme of the Sierra Nevada Carnivore monitoring program varying the magnitude of the population decline (20% and 50%), starting population size (N=300, N=150), size of the effective sampling area (1, 5, 10, 25 km^2), sampling frequency (annual vs biennial), and the type I error rate. We illustrate the link between abundance and occupancy, showing that a 43% decline in abundance over an 8-year period only resulted in a 23% decline in occupancy, with a 20% decline in abundance resulting in a 6% decline in occupancy. This indicates that most sampling schemes may only be able to detect large magnitude declines in abundance using occupancy modeling. We found that given an average annual sample size of 140, over an 8 year sampling period this sample size had a 64% power to detect a 20% decline in occupancy with 20% statistical certainty. Concentrating resources and sampling a larger number of sites biennially instead of a smaller number annually decreases the standard error of the occupancy estimates and results in an increase in statistical power. Our results show that increasing the effective sampling area, implementing biennial instead of annual sampling, and increasing the type I error rate all increase statistical power to detect trend.

Introduction

One of the most fundamental questions for the conservation of a species is "what is the status of the species"? The answer to this question is a critical component of the listing and recovery of threatened and endangered species. Yet, this seemingly basic question of whether a population is increasing, decreasing, or stable can be very difficult to answer. Obtaining accurate estimates population abundance, especially for rare species distributed across large landscapes, is logistically difficult and expensive (Karanth & Nichols 1998; Kendall *et al.* 2008). Consequently, repeating such studies over long time periods (i.e. population monitoring) in order to estimate a trend in population abundance is often cost prohibitive. Estimating trend using occupancy (Ψ), the proportion of an area occupied by a species, has emerged as a more cost effective alternative to estimating trends in abundance for many population monitoring programs (MacKenzie & Nichols 2004; MacKenzie *et al.* 2006).

The underlying assumption in monitoring for population trend using occupancy is there is a positive relationship between the two such that a change in occupancy is indicative of a change in population size (MacKenzie & Nichols 2004). This positive relationship has been well documented, but the shape and strength of the relationship will vary depending on the specific characteristics of the species or population such as the density or spatial aggregation of individuals (Hartley 1998; Gaston *et al.* 2000). Therefore, in some populations a large change in abundance may have a negligible impact on occupancy while in other populations a relatively small change in abundance may result in a substantial change in occupancy. A difficulty in monitoring occupancy as a surrogate for abundance is that for most populations the nature of this relationship is unknown.

Power analyses are an essential element of population monitoring programs. The goal of a power analysis is to establish the ability of any given sampling strategy to detect a change in a population metric and help researchers determine the sample size needed to detect this trend with an acceptable level of statistical confidence. While a number of site specific factors may affect power, it is well established that, in general, statistical power is dependent on: 1) effect size (magnitude of change in a population metric), 2) the variance in population metrics, and 3) sample size (Steidl *et al.* 1997). Estimating these terms can be very difficult, especially when using a surrogate measure such as occupancy and may depend on many population specific variables, such as landscape characteristics, movement rates, and territoriality behavior. Furthermore, power analyses are complicated by the balance of type I versus type II error rates (Mapstone 1995). Type I error rates determine the probability that a method identifies a trend where none really exists, which we refer to as statistical certainty or significance. Type II error rates determine the statistical power of a method to identify a trend that does exist. Thus, power represents the percent of times a method identifies a trend under a given significance threshold when a trend exists. It is the job of the scientist or wildlife manager to weigh the importance of these interrelated components for their particular population in developing a monitoring strategy.

In 2002 the U.S. Forest Service initiated a long-term monitoring program to determine the population status and trend of fisher fisher (*Pekania pennanti* [Kopefli *et al*. 2008, Sato *et al.* 2012], formerly *Martes pennanti* [Erxleben 1777]) in the southern Sierra Nevada of California (Zielinski & Mori 2001; Zielinski *et al.* 2013). There are acute conservation concerns for this population due to its small population size (Spencer *et al.* 2011) and complete geographic and genetic isolation (Knaus *et al.* 2011; Tucker *et al.* 2012) It is currently a candidate for listing as a distinct population segment under the Endangered Species Act (U.S. Department of the Interior, Fish and Wildlife Service 2004). The need for a monitoring program was precipitated by a major amendment to the National Forest Plans in the Sierra Nevada in 2001 that included new prescriptions for forest management designed to address the risk of high severity fires and as well as mandates to monitor the impacts of these proposed treatments on wildlife populations

(U.S. Department of Agriculture 2001; U.S. Department of Agriculture 2004; Zielinski *et al.* 2013).

Prior to implementing this monitoring program a power analysis determined a sample size of 288 units/year, for 10 years, was needed to detect a 20% decline in occupancy (1-sided alternative hypothesis) with a type I error rate of 20% and 80% power (Zielinski & Mori 2001). However, implementation of the monitoring program revealed that this annual target sample size was unattainable given logistical and financial constraints. After eliminating dangerous and extremely remote sites, the monitoring program was reduced to a core set of 223 sample units. An analysis of the first 8 years of data from this monitoring program (2002-2009), in which an average of 139.5 units were sampled per year, found no trend or statistically significant variations in occupancy and concluded that the southern Sierra fisher was not decreasing (Zielinski *et al.* 2013). However, the power to determine trend in occupancy was not formally revisited despite the reduced sample size compared to the targeted sample size (~140/year vs. 288/year).

In this analysis we use a spatially based simulation approach that accounts for the specific natural history and habitat characteristics of the Sierra Nevada fisher population, to model a declining population (Ellis *et al.* In Review). We then mimicked the sampling regime of the actual fisher monitoring program over 8 years to 1) investigate the relationship between occupancy and abundance and 2) reassess the power of the monitoring program to detect trend in occupancy, and 3) determine how power is affected by variations in sample design and statistical confidence. We chose to conduct our simulations over an 8 year period to facilitate comparison to the work of Zielinski *et al.* (2013).

Methods

Fisher monitoring program: study area and methods

The study area is defined as the west slope of the Sierra Nevada from Yosemite National Park south to the end of the Sierra Nevada at Lake Isabella. The majority of sampling (92%) occurred on National Forest lands. Sampling units for the fisher monitoring program are collocated with the Forest Inventory and Analysis (FIA) sampling grid (Bechtold & Patterson 2005). The FIA grid is composed of hexagonal grid cells 24.28 km^2 in size, with the centers of each grid cell 5.3 km apart. FIA vegetation sampling points are randomly located within each of these grid cells, and the center point of each fisher sampling unit is off-set 100 m from a FIA point. To establish potential sample units for the fisher monitoring program, FIA points in habitats known to be unsuitable or outside the possible elevation range (below 800m, above 3200m) of fisher were eliminated, resulting in 388 potential sampling units.

Sample units consisted of an array of six track-plate stations deployed in a pentagonal arrangement (one center station surrounded by five perimeter stations) that encompasses a 0.8

km^2 area (Figure 5-1). The use of bait (chicken) and a commercial trapping lure increases the effective survey area by an unknown distance (Schlexer 2008). Positive identification of fisher from track data has been well established (Zielinski & Truex 1995). Each PSU is deployed for 10 days and checked every 2 days during this period. When stations are checked all tracks and hair samples are collected and track plates, hair snares, and bait are replaced so that each check acts as discrete capture session.

The original sampling plan was to sample half of these units each year such that 388 units would be completed in a two-year period. However, this goal turned out to be logistically unfeasible. Instead an average of 139.5 units were completed each year although sample size per year varied widely (range 90-189) over the first 8-years. As described above a large number of units were eliminated due to safety concerns because they were extremely remote and difficult to access (>14km from a road) resulting in a core set of 223 units. Further details regarding the sampling methods of the fisher monitoring program are described in the Sierra Nevada Forest Plan Amendment Carnivore Monitoring Protocol (Truex & Tucker 2006) and Zieinski *et al.* (2013).

Spatially explicit power analysis

Details of the spatially explicit simulation approach we used are described in full in Ellis *et al.* (In Review). In summary, this approach involves three parts. First we simulated a declining population of fisher in the Sierra Nevada by defining a landscape representing fisher habitat and then distributing individuals spatially across this habitat. We then simulated population declines by removing individuals each year over a 10 year period. Second, we defined a sampling grid and used this grid to sample our simulated landscape and create presence-absence encounter histories (Figure 5-2). We then used these encounter histories to obtain estimates of detection probability and annual occupancy. In the third step we fitted a trend model to these annual occupancy estimates and estimated statistical power as the percentage of simulations in which the declining trend was detected. All spatial simulations were conducted using program SPACE (Spatially-based Power Analyses for Conservation and Ecology) (Ellis *et al.* In Review).

Defining the landscape

We used existing probability of use models for fisher in the southern Sierra Nevada to define where fishers occurred in this landscape. The simulated landscape for fisher was defined primarily by the probability of occurrence model described in Spencer *et al.* (2011) which was based on a generalized additive model of environmental variables correlated with the detection-non-detection data from the Sierra Nevada fisher monitoring program from 2002-2006. As the spatial extent of this model did not completely encompass the area we were interested in simulating, we used the probability of use models described in Davis *et al.* (2007) to complete our simulated landscape in areas not covered by the Spencer *et al.* (2011) model.

Distributing individuals on the landscape

We distributed individuals across the landscape relative to the probability of use with a cutoff of >0.30. We chose to use a cutoff of 0.30 because values less than this seemed to overestimate fisher occupancy, placing individuals in areas where fishers have not been detected in recent history. To distribute individuals across the simulated landscape we randomly selected points within the landscape and weighted these points by probability of use to define home range centers. Home range sizes for fisher in the Sierra Nevada have been shown to differ by sex (Mazzoni 2002; Zielinski *et al.* 2004) and so we defined separate home range sizes for males (42.6 km^2) and females (10.4 km^2). These home range sizes were estimated as an average of home ranges sizes reported for two different study areas within the study area (Zielinski *et al.* 2004; Sweitzer Unpublished Data). Because the Sierra Nevada fisher population has been shown to be female biased (Jordan 2007) we placed female home ranges on the landscape in greater proportion than males (60% females: 40% males). Fishers are intrasexually territorial, but home ranges may overlap between the sexes (Powell 1993) so we established the buffer distance between home range centers equal to 1.5 the radius of the home range to allow partial overlap of home ranges of both males and females. Locations of male and female home range centers were added iteratively until the target population size was reached. We simulated two different starting population sizes, N=300, which has been estimated to be the maximum adult population size for the Sierra Nevada population (Spencer *et al.* 2011), and N=150 to investigate how population size and density affect the abundance/occupancy relationship and statistical power (Figure 5-2).

Individual utilization distributions and population declines

After establishing home range centers, each individual was assigned a bivariate normal utilization distribution based on estimated parameters defining spatial use within a home range. Because each fisher monitoring sample unit is completed within a 12-day period, we felt the potential for long distance movements during such a short time period was low. Therefore, we assumed that individuals spend 90% of their time within their home range radius, and truncated their movement to within 1 SD of their home range center. We used these parameters as the basis for a bivariate normal utilization distribution and also weighted movements by the probability on use on the landscape. We standardized each of the distributions into a probability surface in which individuals have a decreasing probability of use as the distance from the home range center increases, conditioned on probability of use of the landscape. Finally, we combined the individual utilization distributions across N=300 or N=150 individuals in the population to produce a probability surface describing the probability of at least one fisher being present at any point on the landscape. We used these methods to create 250 surfaces each for populations of N=300 and N=150 individuals. We then simulated a 20% or 50% decline in population size in an exponential fashion over a 10-year period ($\lambda = 0.977$, $\lambda = 0.933$) by randomly removing a fixed number of individuals successively over each time step from each of these 250 landscapes.

Sampling

We created a 25km^2 sampling grid, excluding cells that did not overlap our probability of use surface by at least 50%. This resulted in a grid with 358 cells available for sampling. We created encounter histories (estimated presence or absence within a sampling area for each visits) for each simulated landscape in order to estimate occupancy across a gridded landscape. Our simulation was designed to closely match the sampling methods implemented from 2002-2009 by the Sierra Nevada fisher monitoring program (Truex & Tucker 2006; Zielinski *et al.* 2013).

Because the distance from which the lure and bait attract fisher to the sampling devices is unknown we tested a range of effective sampling areas (the area within each 25km^2 that is actually sampled). We distributed random sampling points within each cell to define the center of the effective survey area within each cell. We then started by setting the effective sampling area to 1km^2, which is representative of the area encompassed by the sampling devices plus an additional 150 m buffer beyond the detection device to account for the effect of the bait and lure. We then tested 3 successively larger effective sampling areas of 5km^2 (~950 m lure distance), 10km^2 (~1500m lure distance), and 25km^2 (entire grid cell, ~2750m lure distance). We then used SPACE to calculate the probability of at least one fisher being present in each sampling area (probability of presence) and created 10-year encounter histories for each landscape from which we estimated occupancy. Specifically, we calculated the probability of at least one fisher in each cell on the landscape by overlaying the individual utilization distributions and assigning a 1 (present) or 0 (absent) for each visit by comparing a random draw from Uniform (0,1) with the probability of presence for that cell. Draws less than the probability of presence resulted in a "presence", and a 1 in the encounter history for that visit. Thus, a cell with simulated encounter history "010" indicates that 3 visits were made to the cell in a given year, and fisher were present in the cell only during the second visit.

Detection probability

The simulation is designed such that an individual's home range can span multiple cells. Therefore, a single individual can result in a 'presence' being detected in two or more adjacent cells during the sampling period. This violates an important assumption of occupancy estimation that the system remain closed during sampling (i.e., the occupancy status of a cell cannot change during the sampling period). Because of this viotation of assumptions, the interpretation of estimated occupancy parameters is different than the more standard interpretation of these parameters when the status of a given cell (occupied or not) is fixed during sampling.

The estimate of occupancy under this assumption is the probability that any given cell is used rather than occupied. Consequently, for our simulation, occupancy actually refers to the probability of use. Detection probability is therefore a product of the true detection probability (probability of detecting an individual if they are there) and the probability of presence (probability an individual is present and available for detection). For this study the actual

detection probability specified by the simulations is referred to as p_{sim}. We refer to the per visit detection probability estimated by the model as p_{est} with $p_{est}= p_{sim}$ x probability of presence. Across multiple visits, we can calculate a cumulative detection probability (p_{total}) as $p_{total}=1-(1-p_{est})^{n\_visits}$. A long history of fisher research in California using track plates has demonstrated that fishers are fairly easily detected using these devices and estimated detection probabilities across multiple visits are generally high (p_{total} =0.71 Zielinksi et al. 2013) and therefore we fixed p_{sim} for all simulations at p_{sim} = 0.8.

Encounter histories

To create encounter histories with imperfect detection we modified the original encounter histories for availability, in which 1 indicates presence and 0 indicates absence, with the actual detection probability specified in the simulations, p_{sim}. For each 1 (presence) in the availability encounter histories, we conducted a second random draw from a Uniform (0,1) and compared it to p_{sim}. The 1 (presence) in the availability encounter history would be retained as 1 (detected) in the updated encounter history if the random draw was > p_{sim}. Values that were either 0 (absent) in the availability encounter history or with random draw < p_{sim} were recorded as 0 (non-detected). We subsampled from these encounter histories to explore how variation in number of visits, and sample size influenced power. Lastly, we examined the effect of biennial versus annual sampling by omitting encounter history data from even numbered years.

Estimating occupancy and trend

Encounter histories for each simulated landscape and parameter set were used as input to program MARK (White & Burnham 1999) using the R package *RMark* (R Development Core Team 2012; Laake & J.L. 2013). Occupancy estimates were obtained by fitting 'Robust Design Occupancy' models in MARK. We then used the variance components procedure to fit a linear random effects trend model to the occupancy estimates. We defined a trend to be detected if 1) the trend was in the correct direction and 2) the confidence interval of the trend parameter excluded zero. Given that there is a known trend in the data, we estimated statistical power as the percentage of simulations in which a trend was detected. We varied the type I error rate to quantify how the choice of statistical confidence level affects power for this study design.

Results

Estimated occupancy and detection probabilities

Estimated occupancy decreased slightly with increasing effective sampling area for the 1km^2, 5km^2, and 10km^2 sizes, (Ψ_{1km}= 0.375, Ψ_{5km}=0.35, Ψ_{10km}=0.32) but was considerably higher when the effective sampling area was equivalent to the entire grid cell size (Ψ_{25km}=0.64). Cumulative detection probability increased dramatically with increasing effective sampling area for all sizes (p_{total_1km}=0.45, p_{total_5km}=0.70 , p_{total_10km}=0.80 , p_{total_25km}= 0.98) (Figure 5-3).

For all effective sampling areas, a smaller initial population size of N = 150, resulted in both lower estimated occupancy and lower cumulative detection probability (for example at 5km^2 effective sampling area, Ψ_{150}=0.26 (0.07 SD), and p_{total} =0.60).

One of the goals of our simulations was to replicate as closely as possible the occupancy and detection probability rates estimated for the fisher monitoring program (Zielinski *et al.* 2013). Both the 5km^2 and 10km^2 simulations resulted in estimates comparable to Zielinski *et al.* (2013) but we felt that the 5km^2 size was a more plausible effective sample area size as it is closer to previous estimates of effective sampling area of 1.2 km^2 (Zielinski & Mori 2001). We dismissed the 1 km^2 effective sampling area due to its very low estimated detection probability, and the 25km^2 effective sampling area due to its very high estimates for detection probability and occupancy. Based on these results we concluded that an effective sampling area size of 5km^2 and initial population size of N = 300 most closely matched the occupancy rate (Ψ=0.367) and detection probability (p_{total} = 0.71) found by Zielinski *et al.* (2013) and we used these value as our baseline for subsequent analyses.

Comparison of occupancy and abundance

We were interested in the ability to detect a 50% (λ = 0.933) or 20% (λ = 0.977) decline in abundance over a 10-year period through occupancy modeling. Over the 8-year sampling period, λ = 0.933 lead to a decline in abundance from N=300 to 172 individuals (43% decline) and λ = 0.977 corresponds to a decline from N=300 to 249 (17% decline). These trends did not directly translate to equivalent declines in occupancy. When assuming a 5km^2 effective sampling area, a decline from N = 300 to N = 172 over an 8-year period (λ=0.933) translated to a decline in occupancy from 0.35 +/- 0.05 (SD) to 0.27 +/- 0.07 (e.g. a 23% decline in occupancy), while a 20% decline in abundance from N = 300 to N = 249 resulted in a decline in occupancy from 0.34 +/- 0.06 to 0.32 +/- 0.06 (e.g. a 6% decline) (Figure 5-4A). Therefore, with a starting population size of 300, a 20% decline in occupancy is most comparable in our simulations to a 50% decline in abundance. When simulations were conducted using a smaller initial population size of N = 150, a 50% decline in abundance resulted in a 27% decline in occupancy from 0.26 (+/- 0.07) to 0.19 (+/- 0.07) while a 20% decline in abundance resulted in an 11% decline in occupancy to 0.23 (+/- 0.07) (Figure 5-4B).

Statistical confidence

Power to detect trend was strongly influenced by the choice of the statistical confidence (type I error rate) used for occupancy estimates (Figure 5-5). Decreasing the statistical confidence level caused a considerable increase in power, and this increase in power was most profound at small sample sizes. Our simulation that was most comparable to the Zielinski and Mori (2001) power analysis (80% one tailed CI) found very similar results, with an annual sample of 288 cells yielding 85% power to detect a 50% decline in abundance (~ equivalent to a 23% decline in occupancy) (Figure 5-6). However, if a more conservative two-tailed 95% confidence interval is

used as a significance criteria then the power for a sample of 288 cells decreases to 71%. While the target sample size established in the power analysis was 288, due to logistical difficulties the average annual sample actually completed by the monitoring program from 2002-2009 was ~140, less half the initial target. In our simulations a sample size of 140 cells resulted in a power of 64% using a one tailed 80% CI, and 28% using a two tailed 95% CIs, a much larger loss of power between the two confidence levels than was observed for the larger sample size. Even using the larger sample size of 288, power to detect a 20% decline in abundance (6% decline in occupancy) was poor for both confidence levels (power at 80% CI = 58%, power at 95%CI = 39%).

Non-continuous sampling

We investigated the impact of biennial sampling on power (i.e. completing a sample of 280 every other year) instead of 140 per year in each of two years. We found a biennial sample of 280 yields 71% power using a one tailed 80% CI, and 51% using 2 two tailed 95% CI, an increase in power over an annual sample of 140 of 7% (80% CI) or 23% (95% CI) (Figure 5-6).

Effective Sampling Area

The size of the effective sampling area within the 25km^2 cells, determined by the attractiveness of the lure, had consequences for the power to detect a declining trend. Increasing the effective sampling area from 5 km^2 to 10km^2 increased the power by 10% for an annual sampling regime of 140 cells. For a biennial sample of 280 the power at 10km^2 increases to 76%. For simulations in which the effective survey area equaled the size of the survey gird (25km^2), and estimated detection probabilities approached 1.0, the power for either an annual sample of 140 or a biennial sample of 280 approached 100%.

Discussion

Determining the status and trend of a population is a fundamental component of population management, and the answer to this question is a key factor in the listing and recovery of endangered species. The goal of population monitoring programs is to answer this question, and *a priori* power analyses provide a way to assess the statistical power of different sampling strategies to detect population trends. However, for populations spread across large landscapes, or for species that are rare or difficult to sample, it is often challenging or to achieve the sample sizes identified by these power analyses. In such situations careful consideration of the factors influencing power is needed in order to best design sampling strategies find a balance between maximizing power and minimizing the chance of falsely identifying a trend. Using a spatially explicit simulation for we were able to test the ability of an existing population monitoring program for fisher in the Sierra Nevada to detect a population decline using an occupancy modeling approach.

Occupancy vs abundance

Our simulations found that the target effect size of the Sierra Nevada fisher monitoring program, to detect a 20% decline in occupancy, translates to a population experiencing a ~50% decline in abundance over a 10 year period. Simulations of a more moderate population decline (20% decline in abundance) resulted in a change in occupancy of only 6%-11% (N=300 or N=150), an effect size that would be unlikely to be detected by the sample size achieved by the monitoring program. These results indicate that over an 8 year monitoring period the fisher monitoring program as designed will likely be able to detect a population experiencing a severe decline in abundance, but it would not likely be able detect a population experiencing a slower reduction in size. However, our results also show that the relationship between abundance and occupancy is not linear. For a fixed rate of decline in abundance the rate of decline in occupancy increases over time. This non-linear relationship occurs because at high densities more than one individual may occupy sampling area, such that if one animal is lost the sampling area remains occupied. However, as the density of animals on the landscape decreases an increasing proportion of sampling cells are occupied by only one individual such that it becomes increasingly likely that a loss of individuals will result in a reduction in occupancy. This has important implications for long term population monitoring as it illustrates that when estimating trends in occupancy the longer the timespan a monitoring program samples a population, the more power it will have to detect slow population declines.

Factors affecting statistical power

Our results show that the annual sample size achieved by the fisher monitoring program (~140 units) resulted in a 64% power to detect to a 20% decline in occupancy with 80% power, and a 20% type 1 error rate, a reduction in power of 21% compared to the target sample size of 288 identified in the 2001 *a priori* power analysis (Zielinski & Mori 2001). If the sampling of the fisher monitoring program is analyzed as a biennial sample of 280 units, then the power increases to 71% for an equivalent power and type 1 error rate. This is because concentrating resources and sampling a larger number of sites biennially instead of a smaller number annually decreases the standard error of the occupancy estimates and results in an increase in statistical power. Assuming a fixed cost per sample unit this indicates that a monitoring program can potentially increase its power to detect trend by concentrating it resources to conduct a large biennial sample instead of more frequent but smaller annual samples. However this strategy may prove logistically unfeasible for many monitoring programs, especially those sampling large and remote landscapes. For example, completing 280 samples in a single year for the fisher monitoring program would require approximately 50 field technicians sampling continuously for 4-5 months to complete. It is important to note that the fisher monitoring program did not execute a strict biennial sample as simulated, but rather attempted to sample the entire grid within a 2 year period, so the results of the biennial power analysis are only an approximation of this sampling strategy.

It has been shown that the probability of occupancy depends not only on home range size and population density, but also the effective sampling area (Efford & Dawson 2012). The issue how the effective sampling area impacts both occupancy and power to detect trend has rarely been addressed in the literature and has been flagged as a critical issue in study design (Efford & Dawson 2012). Field studies often define their sample unit in terms of grid cell size without addressing how much of that grid cell their chosen method actually samples. It is erroneous to assume that surveying a single point within a large grid cell is representative of the entire cell, and yet it is common for researchers to use the concepts of grid size and effective sampling area interchangeably. In many cases, the effective sampling area is either unknown or variable, i.e., the distance from which lure draws in individuals is unknown and also likely variable due to environmental conditions such as wind, humidity or topography (Schlexer 2008). Our results show that the size of the effective sampling area has a considerable impact on estimates of occupancy, detection probability, and power to detect trend. For example if in our simulations we had not specifically defined the effective sampling area and assumed that it was equal to our chosen grid cell size (25 km^2) we would have estimated that we had near perfect detection probability and our power to detect a decline with 80% statistical certainty (sample size =140) to be almost 100%, much higher than the estimate of 64% we obtained by defining a more realistic effective sampling area. This example illustrates how failing to accurately define the effective sampling area could lead to misleading estimates of power and underestimation of the sample size truly needed to detect a trend.

Our simulations also illustrate the tradeoff between power and statistical certainty (alpha, type 1 error). This relationship exists because increasing the statistical certainty (lowering type 1 error rate), shrinks the confidence intervals for occupancy estimates. As detection of a trend is defined in part by these confidence intervals, the smaller the confidence interval, the less likely it is that these intervals will overlap zero, increasing the likelihood that a trend will be detected. Increasing the sample size similarly decreases the confidence interval and increases ability to detect trends. Consequently, to achieve a desired power you can either increase sample size or increase the type I error rate. For a fixed cost (e.g. a fixed sample size) the researcher must assess the risk associated between type I error (misidentifying trends that do not exist) and type II error (failing to detect a trend when one is occurring) for their species or population (Mapstone 1995; Field *et al.* 2005; MacKenzie & Royle 2005).

This decision is often difficult as in many conservation scenarios the risks associated with committing either type of error are considerable. For example, falsely detecting a trend (type I error) may result in erroneously listing a species and creating unnecessary environmental restrictions that curtail development causing the loss of jobs and economic hardship. Whereas, failing to detect a trend that is occurring (type II error) might lead to the collapse of a species or population (Mapstone 1995). This raises the question of how conservative or liberal to be when planning to monitor a population? For species such as fisher, which exist at relatively low

densities across large landscapes, taking a conservative approach and targeting low type 1 error will result in either 1) very low power to detect trend, 2) a very expensive monitoring program (as a result of the need for large sample sizes), or 3) deeming a monitoring program unfeasible due to this high cost and low power. Our simulation has shown that for fisher, over a 10 year period, large declines in abundance may result in relatively small changes in occupancy. Therefore, if we want to be as certain as possible that we can detect a decline that is in fact occurring, then given the difficulties of sampling such a population, realistically we must accept a higher risk of type 1 error.

Conclusions

Our results illustrate how spatially based power analyses can inform population monitoring. This approach can be used to investigate the relationships between occupancy and abundance by incorporating life history and home range characteristics particular to a species or population, and can help identify the factors driving statistical power for particular study areas and sampling methods. The results of this study demonstrate the complexities involved in determining population trend. In particular, we emphasize the importance of assessing the effective sampling area within the larger sampling grid, as we found that this often-undefined factor can have a large impact on statistical power. Overall, this study illustrates the importance of careful study design and a priori power analyses prior to embarking on a long term monitoring program.

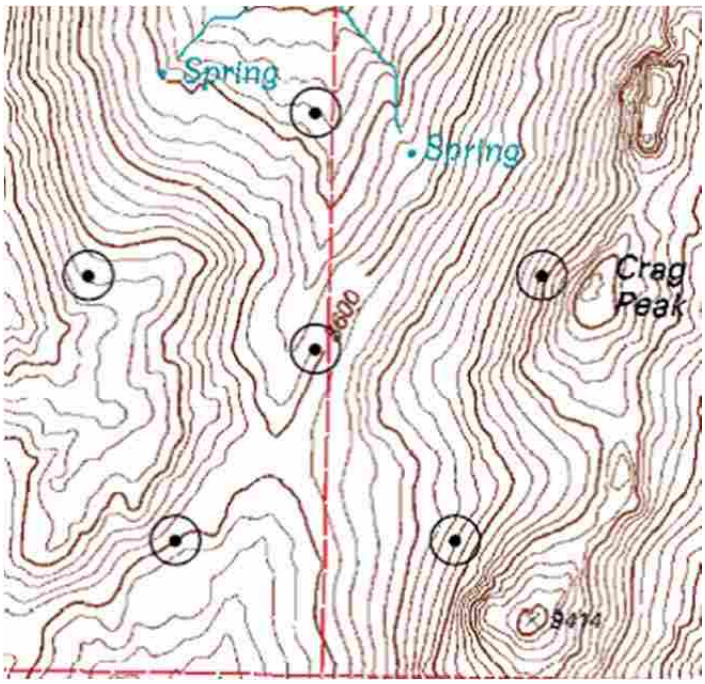

Figure 5-1. Layout of a sample unit for the fisher monitoring program (Zielinski *et al.* 2013). Circles represent 6 track plate stations, each 500 m from the center station.

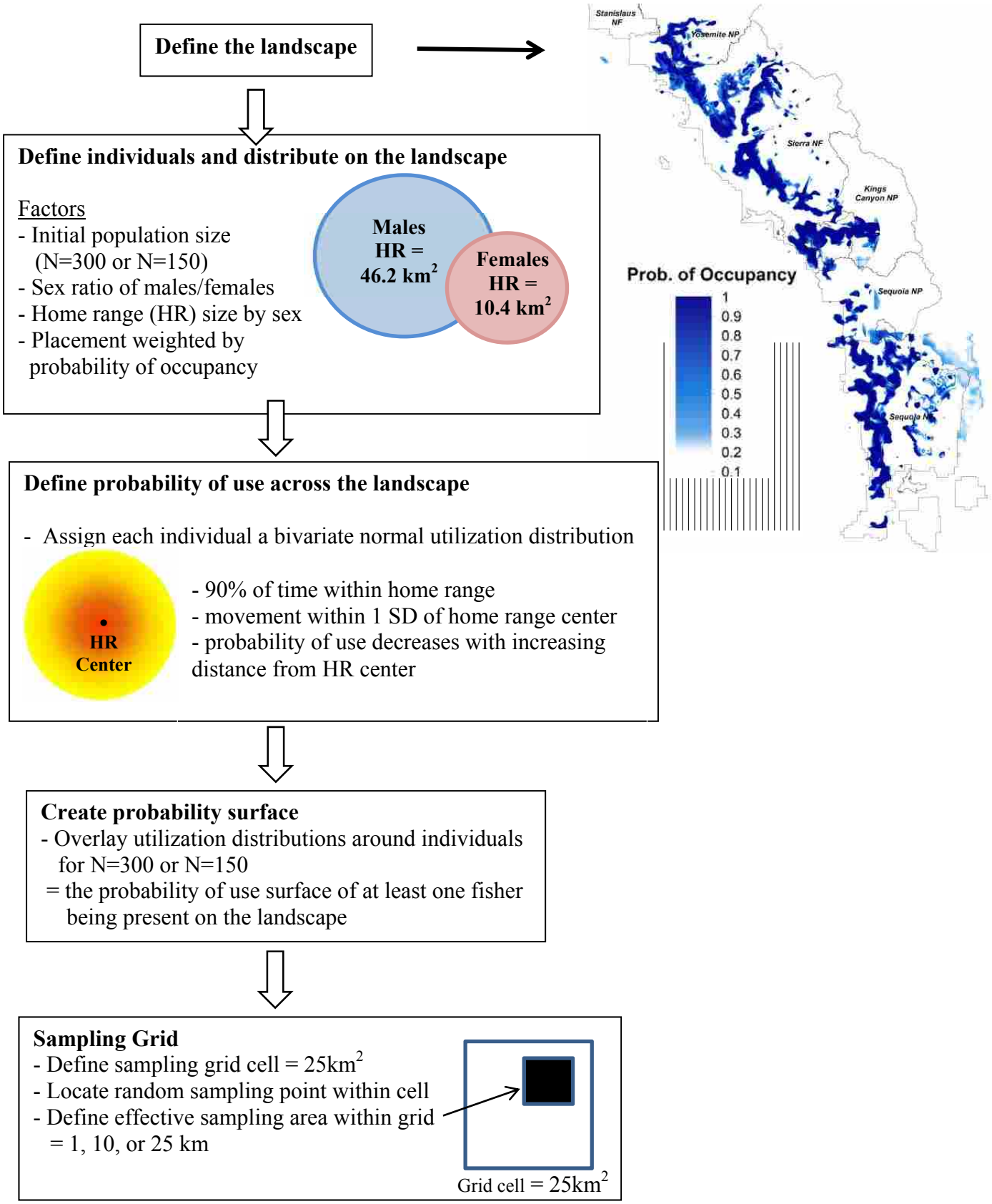

Figure 5-2. Flow chart detailing the spatially-explicit simulation to model fisher occupancy and sampling in the southern Sierra Nevada.

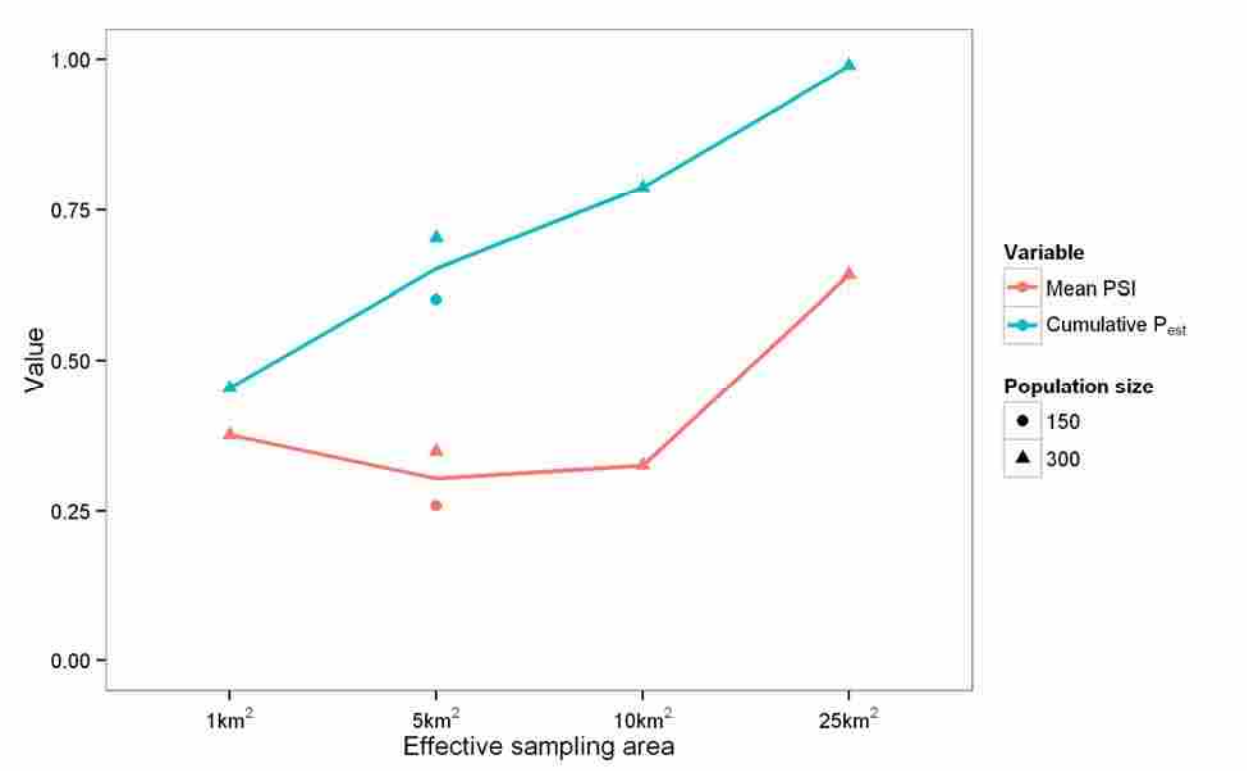

Figure 5-3. Graph showing the relationship between effective sampling area (x-axis) and estimated occupancy (mean PSI) and detection probability (cumulative p_{est}).

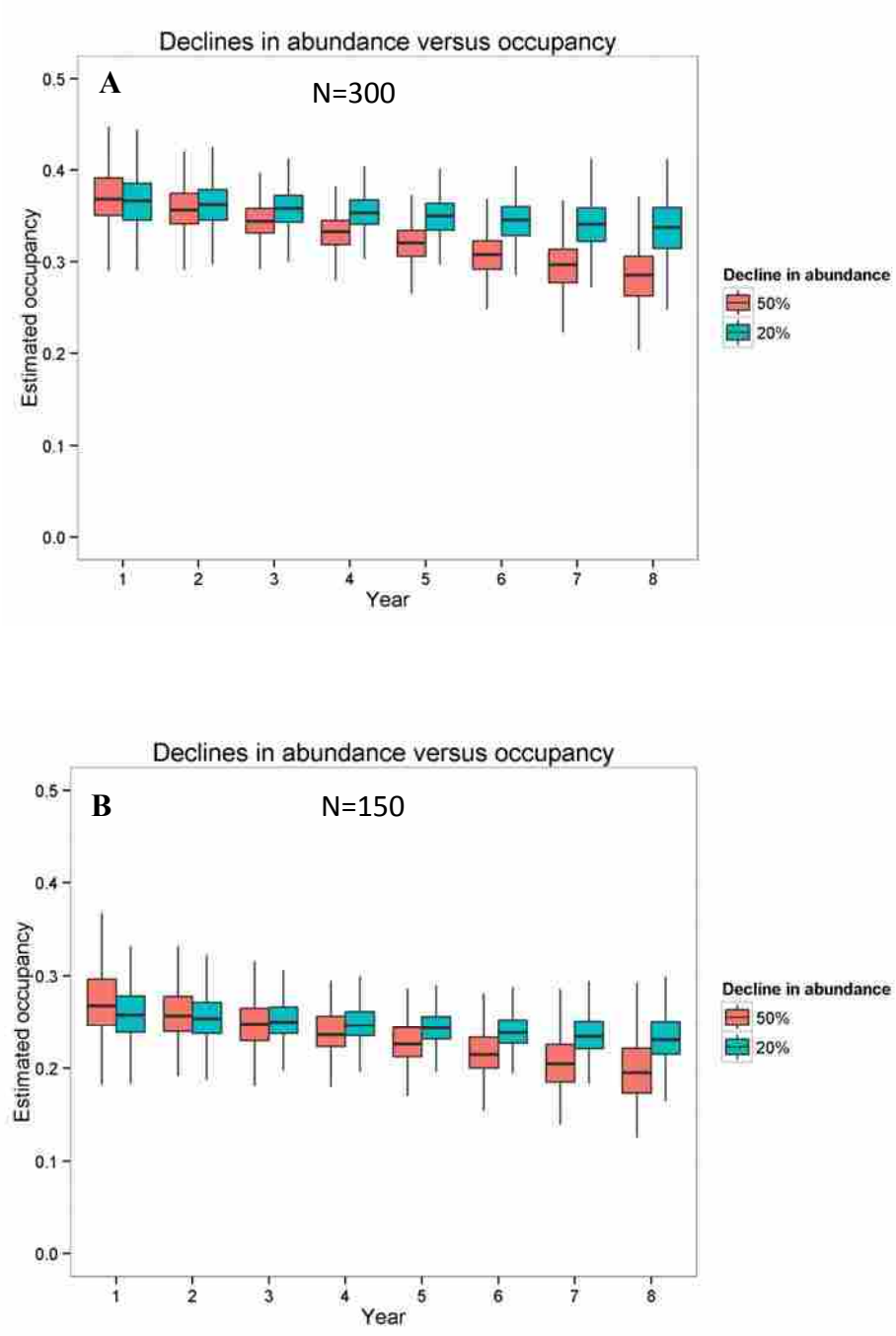

Figure 5-4. Boxplots illustrating the relationship between estimated occupancy and abundance for 250 simulated populations of size A) N=300, B) N=150.

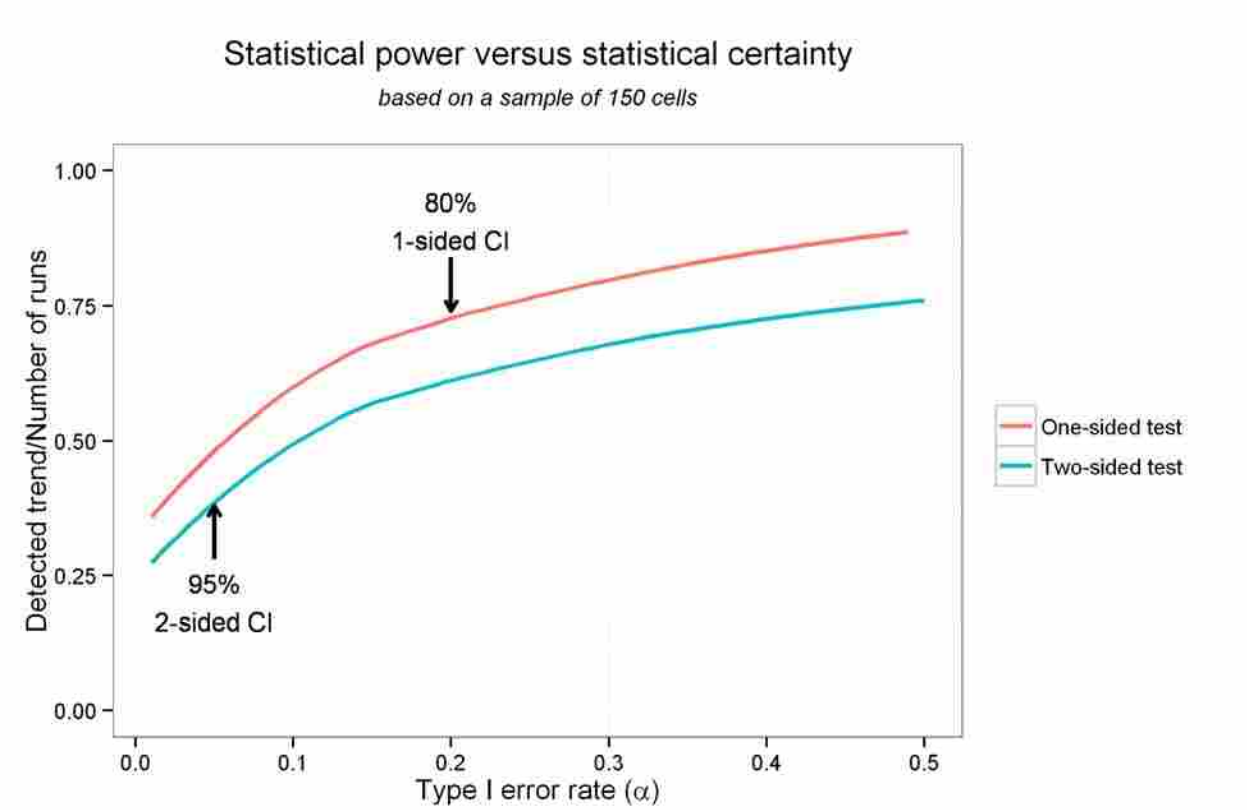

Figure 5-5. Relationship between type I error and power to detect trend for one sided and two sided tests for a sample size of 150. Arrows indicate the power for the two levels of statistical confidence investigated in this analysis.

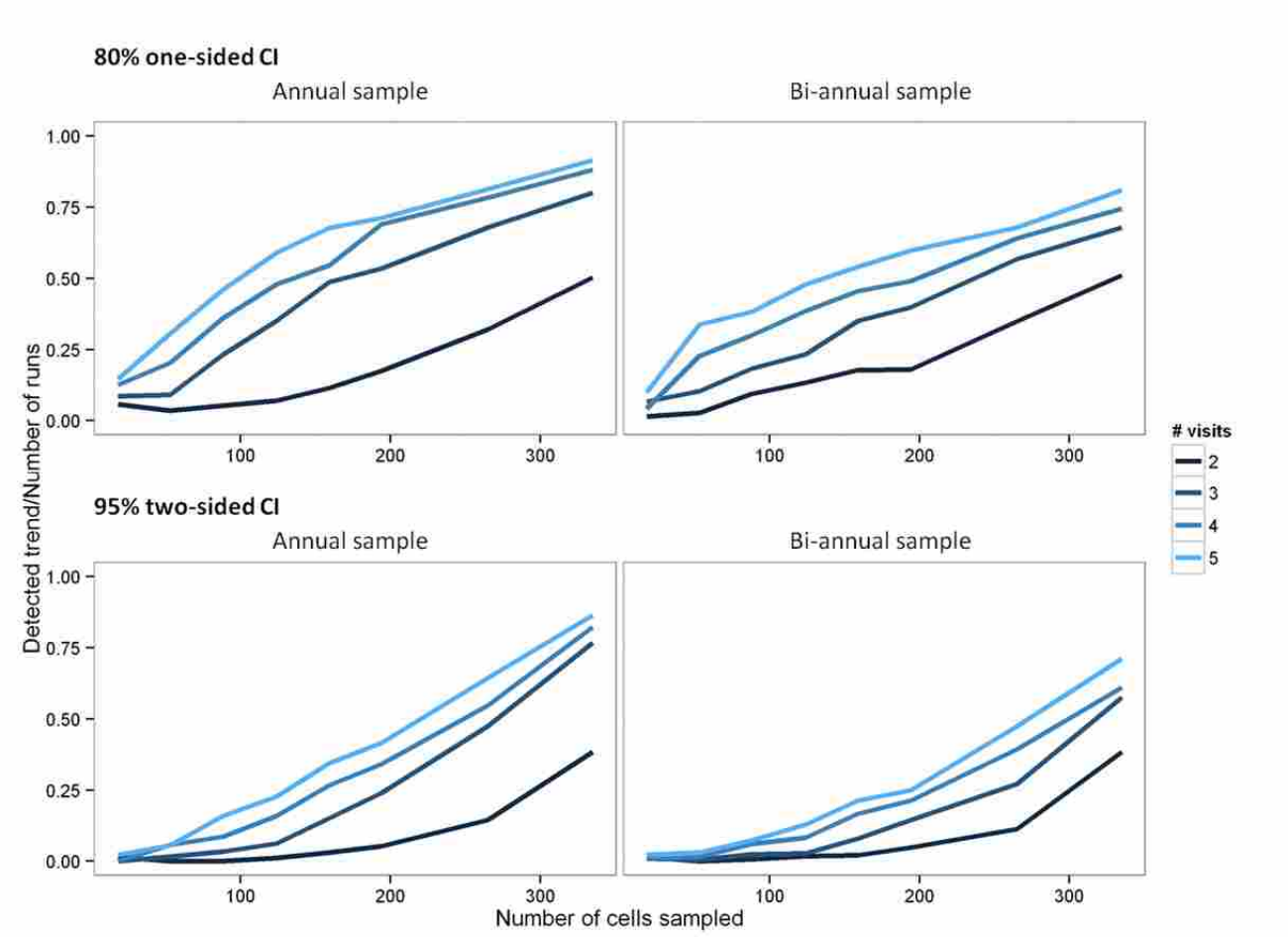

Figure 5-6. Results of spatially explicit power analyses for 250 simulated landscapes with an effective sampling area = 5km^2 and a starting population size of N=300.

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