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POPULATION DIFFERENTIATION AND HABITAT SELECTION OF A MONTANE RED FOX POPULATION IN THE GREATER YELLOWSTONE ECOSYSTEM

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By

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Population Differentiation and Habitat Selection of a Montane Red Fox Population in the Greater Yellowstone Ecosystem.

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Montane red fox (*Vulpes vulpes*) populations across the western United States are genetically and morphologically distinct from foxes at lower elevations. These montane populations also share a preference for subalpine forest habitats. One hypothesis is that they stem from boreal forest-associated ancestors that expanded during the Pleistocene when boreal forests extended farther south than they do today. Forest habitat selection may therefore aid the persistence of native populations surrounded by non-native conspecifics. Alternatively, this behavior may be an avoidance mechanism in response to competition with larger coyotes (*Canis latrans*), or a product of the fox's natural adaptability. The red fox population at high elevations in the Greater Yellowstone Ecosystem (GYE) displays distinctive genetic and morphological characteristics, while it also lives in an environment without resident coyotes. I used genetic analyses to test hypotheses on the origin of this population and to examine population structure and gene flow across the GYE to investigate whether the high elevation population constitutes a discrete and significant population. I also used habitat selection analyses to examine forest habitat selection in this environment and test hypotheses of what may drive this behavior. I found that the GYE serves as a refugium for native red fox genetics, and that forest habitats play a critical role in the life histories of montane fox populations, especially since they hold important food resources used by red foxes such as whitebark pine (*Pinus albicaulis*) seeds. But selection of edge habitats was likewise strong. That suggests that resource scarcity and the need to access a variety of habitats with a variety of resources may be as much of or more important of a driver of habitat selection as are intrinsic preferences or competitive pressures. This project was an application of systems ecology studying how the evolution of a landscape affects the evolution of a species. It analyzed data spanning broad spatial scales−from molecules in DNA sequences to 1 km pixels in satellite remote sensing products−and broad temporal scales−from the minutes between relocations in an animal's movement path to the millennia between glaciations in a geological epoch. Its output benefits the scientific understanding of evolutionary ecology, the management and conservation of native species, and the general public's appreciation of ecology and natural resources. It also addresses whether the population could be considered a distinct population segment under the U.S. Endangered Species Act.

DEDICATION

This thesis is dedicated to Drewyer Pearson (1983 - 2013). Born in Billings, Montana on the 4th of July and named for Lewis and Clark's top hand, Drew was an accomplished biologist, a master angler and natural outdoorsman, a spirited musician, a good hearted troublemaker, and a true friend. He died as he lived, struck by lightning on a sunny day while fly fishing from his canoe high in the mountains of Arizona.

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CRYPTIC SPECIATION ACROSS AN ELEVATIONAL GRADIENT: DETERMINING THE ORIGIN AND POPULATION STRUCTURE OF RED FOX IN THE GREATER YELLOWSTONE ECOSYSTEM THROUGH mtDNA, MICROSATELLITE, AND SPATIAL ANALYSES

INTRODUCTION

Elevational isolation of red foxes (*Vulpes vulpes*) in the western United States has contributed to the development and persistence of locally distinct populations (Aubry 1983, Aubry *et al.* 2009, Fuhrmann 1998, Perrine *et al.* 2007, Swanson *et al.* 2005, Sacks *et al.* 2010). Allopatric speciation driven by alternating vicariance and connectivity during recent ice ages catalyzed this diversity (Aubry *et al*. 2009). Since the 1980s, researchers observing distinctions and similarities in the morphological and behavioral traits of montane fox populations have hypothesized that these local groups are relicts of a once more widespread population (Aubry 1983, Crabtree 1993, Fuhrmann 1998). The recent identification of geographically distinct mitochondrial (mt) DNA sequences with phylogenetic relationships that correspond to the timing and distribution of glacial events strongly supports this hypothesis (Aubry *et al.* 2009).

After diverging from the basal Eurasian population and colonizing North America before or during the Illinoian Glaciation (300,000 - 130,000 years before present), red foxes expanded south into what is today the western United States (Aubry *et al.* 2009). This ancestral population was associated with boreal forests, and after tracking their preferred habitat southward during the glacial advance, they ultimately tracked it upwards in elevation during the glacial retreat, fragmenting the population in isolated mountain

ranges like the Sierra Nevada, Cascades, and Rocky Mountains (Aubry 1983, Fuhrmann 1998). During the following Wisconsin Glaciation (100,000 - 10,000 years before present), this habitat again descended in both latitude and elevation–as much as 1,000 m in elevation in the western United States–reestablishing forest connectivity between the mountain ranges and their respective red fox populations (Volkmann *et al.* 2015). At this time another wave of Eurasian red foxes diverged from the basal population and colonized North America, remaining mostly in ice-free Beringia in what is today Alaska and Northern Canada (Aubry *et al*. 2009). Thus two distinct red fox mtDNA clades, the older Nearctic clade comprising the distinct montane populations and the more recent Holarctic clade farther north, have been present in North America since prehistoric time (Aubry *et al.* 2009).

The first Euroamericans to explore the West took note of its native foxes: in May 1805, Captain Meriwether Lewis shot at, and missed, "the most beautifull [*sic*] fox that I ever beheld, the colours appeared to me to be a fine orrange yellow, white and black... convinced I am that it is a distinct species," while leading the Corps of Discovery on the upper Missouri River in what is now north-central Montana (Lewis *et al.* 1806). Another early description of the "great-tailed fox," as the Rocky Mountain red fox was called until 1936, noted yellowish color tones and a "mixed grizzled gray colour as in the gray fox or badger," (Bailey 1936, Baird 1852, Churcher 1957). Later, anthropogenic translocation of red foxes, including European foxes brought to the East Coast for sport hunting during the late 18th Century and eastern North American foxes brought to the West Coast for fur farming during the early 20th Century, brought non-native red foxes

into contact with these native populations (Kamler and Ballard 2002, Sacks *et al.* 2010, Statham *et al.* 2012).

In some cases, genetic swamping by these expanding non-native populations has caused the extirpation of unique traits in native populations. The Hudson's Bay Company in eastern Canada was among the first to notice this as returns of rarer–and thus more valuable–"silver" and "cross" color phase fox pelts decreased while those of the more common "red" color phase increased (Butler 1945). Yet elsewhere, native populations have persisted despite being surrounded by non-native conspecifics, as is the case with the Sacramento Valley red fox (*V.v. patwin*) (Sacks *et al.* 2010, Sacks *et al.* 2011, Volkmann *et al.* 2015). Sacks and colleagues (2011) suggested that mate discrimination bestowing greater fitness on pure native genotypes than on hybrid genotypes may influence the exclusion of non-native foxes from the native foxes' range.

The existence of distinct local populations within the continuous distribution of a widespread species has likewise been observed in other taxa. Miller (1956) pointed to ecological barriers effecting semi-isolation in the topographically diverse range of the song sparrow (*Passerella melospiza*) to explain the racial diversity of that widespread species. Even greater reproductive isolation is experienced by naturally-hybridizing asters (Asteridae) in the transition between riparian and forest habitat types, where diverging morphological and genetic characteristics prompt a cessation in gene flow (Mitsui *et al.* 2010). Various isolating mechanisms have been identified, including morphological adaptations resulting from changes in foraging strategy or habitat selection, asynchronous reproductive cycles, and both prezygotic barriers like gametic

incompatibility and postzygotic barriers like differing germination times (Lapiedra *et al.* 2013, Lepais *et al.* 2013, Silvertown *et al.* 2005, Knope and Scales 2013).

Cryptic speciation like this presents interesting implications and questions for evolutionary ecology as well as the management and conservation of rare species. Mixing conspecifics from different sources can have both positive consequences, like increasing genetic diversity, and negative consequences, like genetic swamping, outbreeding depression, and disease transmission (Champagnon *et al.* 2012, Roberts *et al.* 2010, Carbyn and Watson 2001). A negative example is found in Wood Buffalo National Park in Canada, where over 6,700 plains bison (*Bison bison bison*) were introduced in the 1920s to supplement its 1,500 woods bison (*B. b. athabascae*) (Carbyn and Watson 2001). This attempt at conservation produced hybrids between the two subspecies and introduced the diseases brucellosis and tuberculosis into the wild population, which continues to be a concern for that region's ecology and agricultural economy today (Carbyn and Watson 2001).

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Red foxes living at high elevations in the Greater Yellowstone Ecosystem (GYE) display distinctive morphological, behavioral, and genetic characteristics (Crabtree 1993, Crabtree 1997, Fuhrmann 1998, Van Etten *et al.* 2006, Swanson *et al.* 2005). The wide variety of coat colors there has been noticed since the earliest days of Yellowstone National Park, where superintendent Philetus Norris wrote in 1881 that the foxes were "numerous and of various colors, the red, grey, black, and the cross varieties (most valuable of all) predominating in the order named," (Fuhrmann 1998, Norris 1881). The

frequency of lighter coat colors significantly increases at elevations above 2,200 m, where pelages with light blond guard hairs and gray underfur predominate over the red pelages more common at lower elevations (Crabtree 1993, Fuhrmann 1998, Swanson *et al.* 2005). Like montane fox populations in the Cascades and Sierra Nevada, those in the GYE display a habitat preference for subalpine forests even though the typical small mammal prey base of this mesopredator is more common in open habitats (Aubry 1983, Fuhrmann 1998, Van Etten *et al.* 2006, Volkmann *et al.* 2015). Swanson and colleagues (2005) also reported significantly greater genetic differentiation between two adjacent populations within the GYE—one above 2,200 m and one below 2,200 m—than there is between this lower GYE population and one in North Dakota more than 1,000 km away.

This contradiction of the genetic structure one would expect from an isolation-by-distance model of gene flow (Wright 1943) led Swanson and colleagues (2005) to suggest that ecological barriers divide the adjacent populations in the absence of geographic barriers. They also supported Fuhrmann's (1998) hypothesis that populations at different elevations in the GYE may have been founded in separate events, and that subsequent selection for traits associated with their respective founders has caused these populations to diverge along elevational lines. According to this hypothesis, instead of being the Nearctic Rocky Mountain red fox (*V.v. macroura*) like those at lower elevations in the GYE, foxes at higher elevations could have descended from Holarctic ancestors that colonized the area during the Wisconsin Glaciation via the Ice-Free Corridor. Linking refugia in Beringia and the south, this corridor periodically opened along the Rocky Mountain Front between the continental Laurentide and montane

Cordilleran ice sheets, and it terminated near the GYE at glacial maximum (Pielou 1991). It may have likewise been used by bison and even humans colonizing North America (Kashani *et al.* 2012, Wilson 1996). Alternatively, this population may have been founded much more recently by the expanding non-native population (Kamler and Ballar 2002, Statham *et al*. 2012).

Therefore, the primary goal of this study was to determine whether the red fox population living at high elevations in the GYE constitutes a discrete and significant population unit, thereby gaining insight on the origin of the population as well as the mechanisms facilitating its persistence in modern times. I predicted that it was either a completely distinct population associated with the Holarctic clade, a distinct branch of the Nearctic clade, a recently arrived non-native population, or not significantly different from surrounding foxes at all. Specific objectives included:

1. Determining the historical phylogenetic relationships of foxes sampled in and around the GYE using the same mtDNA sequences that Aubry and colleagues (2009) studied in historical (> 100 years old) fox specimens sampled worldwide.
2. Examining recent gene flow between foxes at different elevations in the GYE using microsatellite data.
3. Examining whether sex-biased gene flow impacts the patterns of differentiation observed using measures of differentiation for both mtDNA and microsatellites.
4. Assessing the correlation of genetic variance and geography by plotting the spatial distribution of genetic variants with geographic information systems (GIS) and with logistic regression.

The first objective tests Fuhrmann (1998) and Swanson and colleagues' (2005) hypothesis that the high elevation GYE population was founded by Holarctic ancestors, thereby determining whether it is a significant historic population. The second objective assessed its connectivity with surrounding populations, thereby determining whether it is a discrete population, as well as private alleles that are evidence of long-term isolation. The third objective examined a mechanism suspected of affecting population differentiation in the GYE, while the final objective examined the effects of elevation and topography on population differentiation there.

The results of this study indicate that the GYE serves as a refugium for native red fox genetics, which may in part be due to asymmetric gene flow with respect to gender across the elevational gradient.

MATERIALS AND METHODS

Study Area

Genetic data was collected in the GYE from three elevation groups: high (2,700 m - 2,900 m), middle (2,000 m - 2,200 m), and low (1,100 m - 1,500 m). The high elevation area is above the 2,200 m threshold where the frequency of lighter coat colors significantly increases (Swanson *et al.* 2005). It is centered at Beartooth Lake (44.9446ºN, 109.5890ºW) in the Shoshone National Forest, Wyoming, USA. The land cover there is predominantly subalpine fir, spruce, and whitebark pine forests with xeric and mesic meadows. The middle elevation area is centered on the Lamar Valley (44.8975ºN, 110.2560ºW) in Yellowstone National Park, Wyoming, USA. The land cover there is predominantly xeric, mesic, and sagebrush meadows with lodgepole pine and douglas fir forests. It's center is approximately 55 km west of the center of the high elevation area. The low elevation area surrounds the high and middle elevation areas, falling below an 1,800 m elevation threshold and within a 150 km radius of the high elevation area. It includes agricultural and other developed lands, semi-arid grasslands, sagebrush foothills, and lodgepole pine and douglas fir forests. The mean distance of low elevation samples from the center of the high elevation area is approximately 80 km, the closest being 40 km to the east and the farthest being 116 km to the northwest.

Trapping and Sample Collection

The high elevation group (n = 9) was sampled primarily through live trapping using steel leghold traps, plywood box traps, and log cabin traps over a two year period

(Copeland *et al.* 1995, Fuhrmann 1998, Van Etten *et al.* 2007). Traps were spaced no more than 2 km apart on a 12 km trapline. I expected to target eight territories/fox families and up to 25 individuals, assuming a continuous distribution of 4 km^2 territories each with a resident breeding pair and one "helper" yearling female in each territory (Crabtree and Sheldon 1999, Hersteinsson and Macdonald 1982, Fuhrmann 1998). Observations of territorial scent marking behavior, plus the home range analysis of a VHF collared male fox in the area, helped identify territorial boundaries and guide the placement of traps near those boundaries.

Numbers 1.5 and 3 soft-catch, center swivel, padded steel leghold traps with offset jaws (Woodstream Corp., Lititz, PA) and plywood box traps measuring 0.46 m wide, 0.6 m high, 1.22 m long (Keith Van Etten, Cooke City, MT) were used from May 30, 2012 to June 19, 2012 (308 trap nights). Log cabin traps built on site were used from February 2, 2013 to May 10, 2013 (115 trap nights) and from January 9, 2014 to April 2, 2014 (173 trap nights). These are effective in the winter since they hold large quantities of meat for bait, making them attractive to food-stressed foxes but not to dormant bears, and they safely restrain captured animals without contact or exposure. I allowed more space between logs than in the trap designed by Copeland and colleagues (1995) to capture wolverine (*Gulo gulo*). Foxes are less aggressive than wolverines and do not try to chew their way out of a log cabin trap like wolverines do (Keith Van Etten, personal communication), while the gaps allowed American marten (*Martes americanus*) bycatch to escape on its own.

Foxes were restrained with a noose pole and padded Y-pole, chomp bit and

electrical tape securing the muzzle, blindfold, electrical tape securing the paws, and a heavy blanket (Van Etten *et al*. 2007; Keith Van Etten, personal communication). A sedative approved for a 4 kg animal−1 cc of a 10:1 ketamine:xylezene blend−was available but rarely used as the restraining method allowed processing without anesthetics. The animal identification number, gender, weight, age estimate (based on tooth wear, weight, and teat condition), and trap location were recorded. A tissue sample was collected for genetic analysis, and the fox was fitted with a radio collar for habitat selection analysis before being released.

Tissue samples were collected with an ear punch, preserved in ethanol or silica desiccant, and stored in a cool, dark place prior to DNA extraction.

We captured eight individual foxes in the high elevation group, including two adult males, two adult females, one subadult male, and three subadult females. An additional sample from a female fox captured in the high elevation area (H4) was volunteered by fur trappers. All of these samples yielded usable DNA. Interestingly, all of the 2013 captures occurred in March at traps baited with elk while those baited with deer were ignored. Yet in 2014, all of the captures occurred in January and early February at traps baited with deer while those baited with elk were ignored.

Middle elevation samples (n = 10) were collected for a previous study (Van Etten 2006) but never analyzed. They were obtained primarily through live trapping with steel leghold traps and plywood box traps during spring and fall seasons between 2003 and 2005, with the exception of one road kill sample collected in 1998, and included five males and five females. Five of these individuals (M114, M152, M473, F195, and F223)

have additional telemetry and life history information from this previous study. An additional sample (M465) failed to yield usable DNA. All were preserved in silica desiccant and stored in a freezer until DNA was extracted in 2014. When no trapsite data accompanied the sample, the centroid of the animal's telemetry data was calculated and used for genetic sample location data; when there was no data at all for the sample, then the center of the middle elevation group was used for its location data.

Low elevation samples (n = 6) were collected opportunistically from fur trappers, snowplow drivers, and roadkill, providing five males and one female. Preservation protocols were the same as above, and locations were estimated based on landmark descriptions provided. One of these samples (L1), which was obtained from fur trappers in the Sunlight Basin about 24 km south of and across a major canyon from the high elevation area, was later reclassified into the high elevation group since it was determined to be the offspring of a high elevation fox (F100) during genetic analysis.

Laboratory Procedures

Samples were washed (depending on silica or ethanol preservation) and DNA was extracted with a Qiagen DNeasy tissue kit (Qiagen, Holdren, Germany) following the manufacturer's protocol. DNA was successfully extracted from all samples except for one individual from the middle elevation group, and it was amplified at 28 short microsatellite loci developed for red fox from published dog loci (multiplexes 1-4; Moore *et al.* 2010) as well as 354 bp cytochrome *b* and 342 bp D-loop sequences using previously published primers and PCR reactions (Perrine *et al.* 2007; Aubry *et al.* 2009,

respectively). One microsatellite locus in one high elevation sample failed to amplify; all of the rest of the loci and sequences amplified successfully. Laboratory procedures were carried out at the Mammalian Ecology and Conservation Unit at the University of California-Davis Veterinary Genetics Laboratory.

Genetic Analysis

mtDNA

Sequence data from the mtDNA cytochrome *b* gene, which is associated with respiratory function in mammals, and the D-loop control region, which is a non-coding strand of DNA woven into and complementing the cytochrome *b* gene, were compared to previously documented North American and Eurasian haplotypes (Aubry *et al.* 2009). Given their molecular relationship, D-loop haplotypes are typically correlated with a particular cytochrome *b* haplotype, but since the D-loop is a non-coding region it can change more rapidly than the corresponding gene. In wild canines, mutations accumulate on average every 16,473 years on the D-loop compared to every 101,000 years on the cytochrome *b* gene (Aubry *et al.* 2009). Because haploid mtDNA is maternally inherited, it is not affected by genetic recombination like diploid nuclear DNA, making it a useful historical genetic reference. The samples that Aubry and colleagues (2009) analyzed to identify these haplotypes were all greater than 100 years old.

Haplotype and nucleotide diversity indices and the number of polymorphic nucleotide sites in each sample group were calculated with Arlequin 3.5 (Excoffier *et al.*

2005), and pairwise F_{ST} of mtDNA, which was used to estimate female gene flow, was calculated with Genepop 4.3 (Rousset 2008).

Microsatellites

Genotypes were first used to identify related individuals within the sample using ML-Relate (Kalinowski *et al.* 2006), as their inclusion would skew subsequent analyses of population structure. Four sets of first order relatives, including three parent-offspring pairs and one full sibling pair, were detected in the high elevation group (**Table 1.1**). Three half sibling pairs in the high elevation group and four half sibling pairs in the middle elevation group were also detected, and no related individuals were detected in the low elevation group. The offspring individuals (L1 and F306) and one of the full siblings (M500) were removed from the population structure analysis.

Next, I conducted pairwise exact *G* tests between elevation groups using Genepop 4.3 (Rousset 2008). That way I could determine whether the elevational delineators defining each group had ecological significance by considering whether or not the elevation groups were discrete groups of genetic samples. In fact, initial *G* test results showed no significant differentiation between any of the groups, calling into question their validity. Given the similarity in the distribution of mtDNA haplotypes between the high and middle elevation groups (see below), I combined these two groups and repeated the exact *G* test. This resulted in significant differentiation ($p = 0.004$) between the combined high and middle elevation groups ($n = 17$) and the low elevation group ($n = 5$).

Genepop 4.3 was also used to calculate F_{IS} for each locus to detect loci deviating from Hardy-Weinberg proportions (Luikart *et al.* 2003), to calculate allelic richness,

Relate	ID1 : ID2	Cyt. *b*	LnLR	ΔU	ΔHS	ΔFS	ΔPO
PO	M000 : F306	A3:A3	-107.09	10.51	3.88	3.59	~
PO	F324 : F306	A3:A3	-105.61	5.09	1.76	4.12	~
PO	F100 : L1	A:A	-99.41	6.28	2.25	6.82	~
FS	H4 : M500	A3:A3	-115.46	13.52	4.37	~	9999

Table 1.1: ML-Relate (Kalinowski *et al.* 2006) output including relationship (parent-offspring (PO), full sibling (FS), half sibling (HS) or unrelated (U)), IDs, mtDNA haplotypes (Cyt. *b*), log-likelihood of the relationship, and the change in log-likelihood for other relationship estimates.

observed and expected heterozygosity, and linkage disequilibrium within the samples, and to calculate F_{ST} between population pairs, providing a measure of recent gene flow and overall population differentiation. F_{ST} outliers were also identified and examined for their chromosomal allignment and possible linkage to genes under selection.

Private alleles were identified within elevation groups as well as groups of haplotypes. Combined with assessments of maternal ancestry (mtDNA haplotype) and population differentiation (F_{ST}), the existence of private alleles would indicate the significance of a population group since one that has been established for a long time would be expected to have significant frequencies of private alleles.

Finally, I used the microsatellite data to examine population structure across the total dataset, first by analyzing the effect of isolation-by-distance on genetic diversity through individual-based Mantel tests implemented in the R package "adegenet" (Jombart 2008). In this way, the significance of the empirical genetic distance:geographic distance correlation was assessed through 999 Monte Carlo simulations run in the absence of spatial structure. Next, I ran assignment tests using STRUCTURE (Pritchard *et al.* 2000). A series of preliminary STRUCTURE runs was performed with the number of

possible populations (*K*) ranging from one to six, with 10 iterations for each *K* value and 100,000 repetitions following a 100,000 repetition burn-in period for each run to allow the simulation's Markov chain to converge. Statistical measures of model fit like the log probability of the data (L(*K*)) as well as bar plots of the assignments are returned with each run. Runs that failed to converge, identified by L(*K*) values and bar plots that differ substantially from other runs at the same *K* value, were discarded (Faubet *et al.* 2007; Pritchard *et al.* 2000). This resulted in 10%-30% of runs being discarded for each *K* value. The remaining output was then uploaded into Structure Harvester (Earl and vonHoldt 2012) to compute Δ*K*, a function of the standard deviation between iterations for each *K* value and the rate of change in L(*K*) between successive *K* values, using the Evanno method (Evanno *et al.* 2005). The *K* value with the greatest Δ*K* was selected to most likely represent the actual number of populations, while the iteration of that *K* value with the greatest L(*K*) was selected to determine the proportion of genotypes assigned to a given cluster (*q*) for each sample. I established *q* thresholds to distinguish pure genotypes ($q < 0.2$ or $q > 0.8$) from admixed genotypes (Sacks *et al.* 2011).

Sex-Biased Gene Flow

F_{ST} values for both mtDNA haplotypes, representing female gene flow, and microsatellite genotypes, representing overall population differentiation with both male and female gene flow, were used to estimate the ratio of male gene flow to female gene flow. They were applied to Equation 7c by Hedrick and colleagues (2013):

$$\frac{m_m}{m_f} = \frac{(1 - F_{ST})F_{ST(f)} - 2F_{ST}(1 - F_{ST(f)})}{2F_{ST}(1 - F_{ST(f)})}$$

where m_m represents male gene flow, m_f represents female gene flow, F_{ST} is the overall population genetic differentiation value as calculated with microsatellite genotypes, and $F_{ST(f)}$ is the value of genetic differentiation among females in the population as calculated with mtDNA haplotypes. Assuming an island model of gene flow and equal effective population sizes (Ne) between the sexes, results with values greater than one indicate male biased gene flow, whereas results with values between one and zero indicate female biased gene flow. And even though Hedrick and colleagues (2015) caution that this equation is best applied to populations where male gene flow is greater than female gene flow, the substantial difference in median dispersal distances−21.3 km for male foxes and 3.1 km for females (Swanson *et al.* 2005)−suggests that these foxes meet those conditions. This technique has been applied to other GYE species as well, including bison (m_m/m_f = 5.25) and elk (*Cervus elaphus*) (m_m/m_f = 45.9), the latter of which is among the highest ratios reported for large mammals (Hand *et al.* 2014).

Geospatial and Regression Analyses

The geographic distribution of mtDNA haplotypes was plotted using ArcGIS (ESRI, Redlands, CA) to visualize spatial relationships between the distribution of haplotypes and the landscape, while linear relationships between haplotypes and elevation were assessed with the R Statistical Computing Platform (R Core Team 2013). The *q* values from the STRUCTURE assignment tests were likewise plotted with ArcGIS and assessed with simple linear regression models using elevation as the explanatory variable. An additional simple linear regression model of mtDNA haplotype over *q* was also assessed to see if maternal ancestry affected modern population structure.

RESULTS

mtDNA

Cytochrome *b* Haplotypes

Five cytochrome *b* haplotypes were detected in the total dataset, all matching previously documented haplotypes (Aubry *et al.* 2009). Only two haplotypes (A and A3) were found in the high and middle elevation groups (**Figure 1.1**). These haplotypes were historically present in the Rocky Mountains (> 100 years before present) and are thus native haplotypes. Split by elevational group, there were five As and five A3s in the high elevation group, and three As and seven A3s in the middle elevation group.

A different pattern was observed in the low elevation group. Despite the smaller sample size (n = 5), there were four cytochrome *b* haplotypes detected there, which included native and non-native haplotypes as well as haplotypes from both the Nearctic and Holarctic clades (**Figure 1.1**). The two non-native haplotypes included an O haplotype, which is historically from the Cascades, that was sampled about 75 km north of the high elevation study area, and an F haplotype, historically from eastern Canada, sampled about 102 km west of the high elevation study area. The Holarctic N haplotype, historically from Alaska and Canada as well as the Rocky Mountains, was detected in two samples collected about 63 km northeast and 39 km east of the high elevation study area, respectively. Finally, a native A haplotype was detected more than 116 km away from the high elevation study area, the most distant of the low elevation samples.

The number of polymorphic sites in these cytochrome *b* haplotypes ranged from

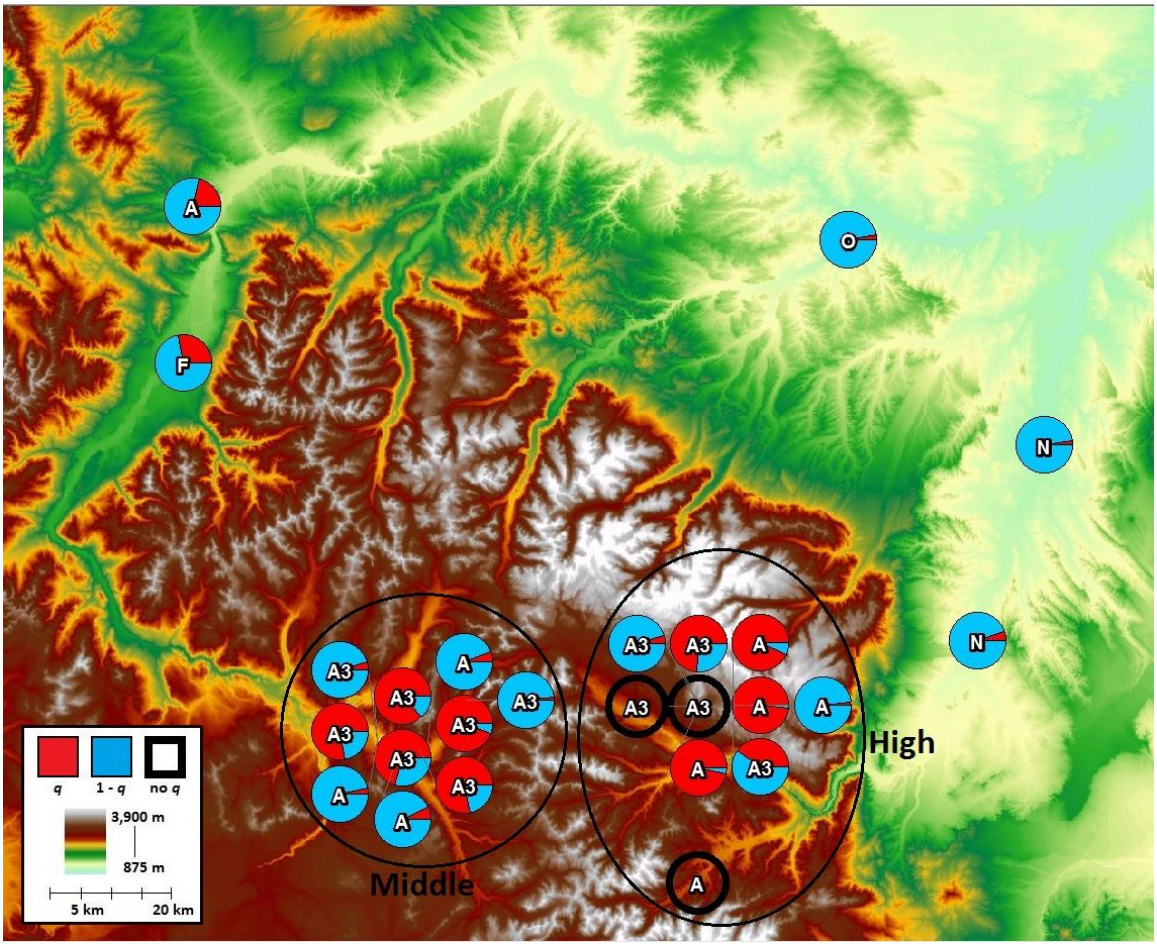

Figure 1.1: Spatial distribution of samples plotted on a 30 m digital elevation model, labeled by cytochrome *b* haplotype and elevation group. Pie charts for each sample display its *q* value and inverse from a *K* = 2 STRUCTURE assignment test. Samples with no *q* value are from first order relatives that were removed prior to assessing population structure as closely related individuals would have biased that analysis.

two in the combined high and middle elevation groups to seven in the low elevation group. Haplotype diversity was likewise lower in the combined high and middle elevation groups (0.5147 ± 0.0592) than it was in the low elevation group (0.9 ± 0.1610).

D-Loop Haplotypes

Five previously documented D-loop haplotypes were detected in the total set along with two novel haplotypes. In the combined high and middle elevation groups, this included the previously documented cytochrome *b*-D-loop combinations A3-59 (n = 9), A-43 (n = 7), and A-19 (n = 1). One novel D-loop haplotype that corresponed with a cytochrome *b* A3 haplotype and differed from the D-loop 59 haplotype by one substitution was detected in the middle elevation group, and it has since been designated haplotype 276 (Ben Sacks, personal communication). In the low elevation group, both the O-24 and F-9 cytochrome *b*-D-loop combinations detected had been previously recorded. The two cytochrome *b* N haplotypes, however, were attached to a novel D-loop haplotype that differed by two substitutions from haplotype 7 and has since been designated haplotype 277.

D-loop haplotype diversity diversity ranged from 0.625 ± 0.0831 in the combined high and middle elevation groups to 0.9 ± 0.1610 in the low elevation group.

Microsatellites

Mean heterozygosity in the combined high and middle elevation groups was 0.7017, compared to 0.65 in the low elevation group, while allelic richness ranged from 6 in the combined high and middle elevation groups to 4.1071 in the low elevation group. One locus deviated from Hardy-Weinberg proportions in the combined high and middle elevation groups (FH2004, F_{IS} = 0.4286, p = 0.1361), and two loci deviated from Hardy-Weinberg proportions in the low elevation group (c01.424PET, F_{IS} = 0.7143, p = 0.0182;

FH2088, F_{IS} = 0.7333, p = 0.0459) (**Figure 1.2**). But none of these F_{IS} estimates were significant ($p < 0.01$), so all were retained in the dataset. Five pairs of loci demonstrated significant ($p < 0.01$) linkage disequilibrium in the combined high and middle elevation groups, but this may have been the result of combining these two similar, but spatially distinct, sample groups, especially given the effect of isolation-by-distance detected (see below). When separated, two pair of loci in the middle elevation group alone continued to demonstrate significant linkage disequilibrium. Since these pairs were only found in one of the groups, they were retained as they may contribute to population structure.

Population Differentiation

Exact *G* Tests

In addition to investigating the elevation groups and, after redefining them, detecting significant differentiation (p = 0.004) between the combined high and middle elevation groups and the low elevation group, exact *G* tests were also used in pairwise comparisons of cytochrome *b* haplotypes assuming that they were divided by reproductive barriers. They revealed significant differentiation (p = 0.006) in the distribution of microsatellite genotypes between the combined A and A3 haplotypes (n = 18) and the combined non-A haplotypes (n = 4). Significant differentiation (p = 0.003) was also detected between the A3 haplotypes alone (n = 10) and the combined non-A haplotypes (n = 4).

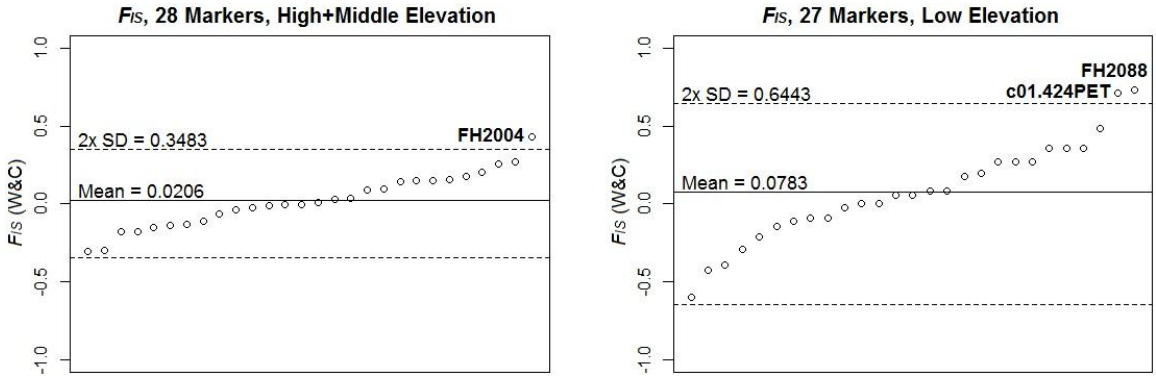

Figure 1.2: *FIS* estimates for each microsatellite locus in the combined high and middle elevation groups (left) and the low elevation group (right). The mean value for each group is indicated with a solid line, and two standard deviations from that mean is indicated with a dashed line. Outlier loci are labeled, however none of these outliers had significant p values ($p < 0.01$) so all were retained in the dataset.

FST Estimates

An *FST* of 0.0259 was calculated using microsatellite data in a pairwise comparison of the combined high and middle elevation groups and the low elevation group. One marker, RFCPH2, was an *FST* outlier as its *FST* was more than two standard deviations greater than the mean *FST* (Table 1.2). Another marker, AHT140, also had an *FST* that was substantialy greater than other markers. Recalculating overall *FST* without these two markers resulted in a much lower *FST* of 0.0138, while the exact *G* test continued to indicate significant ($p = 0.0155$) differentiation between the two groups. Closer examination of the two removed markers revealed that RFCPH2 is on the same chromosome as the *FGF5* gene that controls hair length in canines (Housley and Venta 2006), while AHT140 is on the same chromosome as the *MLPH* gene that affects pale coat colors in canines (Philipp *et al*. 2004).

With haploid mtDNA data, the *FST* was 0.2510: nearly an order of magnitude

Marker	Chr.	Reference	F_{ST}
c01.424PET	1*	Clark *et al.* 2004	-0.0035
FH2848	2	http://www.genomia.cz/en/diverzita	0.0375
RF2457	4	http://cbsu.tc.cornell.edu/ccgr/	0.0001
CPH18	5	http://cbsu.tc.cornell.edu/ccgr/	0.0708
AHTh171	6	http://www.genomia.cz/en/diverzita	0.0817
CPH3	6	Klukowska *et al.* 2002	0.0747
REN162C04	7	Clark *et al.* 2004	-0.0365
RF08.618	8	http://cbsu.tc.cornell.edu/ccgr/	0.0683
AHT142	8	Ruvinsky and Sampson 2001	0.0971
CXX-602	10	Lingaas *et al.* 2001; Neff *et al.* 1999	-0.0005
INU055	10	http://www.genomia.cz/en/diverzita	-0.0389
FH2004	11	Clark *et al.* 2004	-0.0847
AHT137	11	Clark *et al.* 2004	0.0143
RF2054	12	http://cbsu.tc.cornell.edu/ccgr/	0.0014
FH2088	15	http://cbsu.tc.cornell.edu/ccgr/	-0.0701
REN54P11	18*	Clark *et al.* 2004	-0.0438
FH2380	19*	Clark *et al.* 2004	-0.0051
CXX-468	22	Lingaas *et al.* 2001; Neff *et al.* 1999	0.0995
CXX-279	22	http://www.genomia.cz/en/diverzita	-0.0497
RF2001Fam	23	http://cbsu.tc.cornell.edu/ccgr/	0.0964
FH2010	24	http://cbsu.tc.cornell.edu/ccgr/	-0.0384
AHT140	25	McGraw 2004	0.1243
FH2289	27	Wilke 2006	-0.0466
REN169O18	29	http://www.genomia.cz/en/diverzita	0.0472
RFCPH2	*32*	*Clark* et al. *2004*	*0.1829*
FH2328	33	Dayton *et al.* 2009	-0.005
AHT133	37	http://cbsu.tc.cornell.edu/ccgr/	0.004
CPH11	NA	Klukowska *et al.* 2002	0.0188
mean F_{ST}	NA	NA	0.0212
sd F_{ST}	NA	NA	0.065

Table 1.2: Microsatellite markers, their chromosomal (Chr.) allignment on the domestic dog (*Canis familiaris*) genome, and their individual F_{ST} values from pairwise comparison of the two elevational groups. Although the red fox has fewer chromosomes than the domestic dog, most of the dog chromosomes are conserved *in toto* in homologous red fox chromosomes (Yang *et al*. 1999). Those dog chromosomes that are split between two red fox chromosomes are indicated with an asterisk.

greater than that calculated with diploid microsatellite data.

Private Alleles

There were 29 private alleles in the combined high and middle elevation groups, compared to 14 private alleles in the well distributed low elevation group. The high and middle elevation groups individually had 11 and 22 private alleles, respectively. The average frequency of private alleles between the combined high and middle elevation groups and the low elevation group was 0.0840 with a standard deviation of 0.0592. Two private alleles in the combined high and middle elevation groups had significant frequencies (0.25, more than two standard deviations greater than the mean). The average frequency of private alleles between the high, middle, and low groups individually was 0.0324 with a standard deviation of 0.0169. Three private alleles in the middle elevation group and two private alleles in the low elevation group had significant frequencies under these parameters.

With groups of mtDNA (cytochrome *b*) haplotypes, there were 19 private alleles associated with the A haplotype, 19 private alleles with the A3 haplotype, and 13 private alleles for the remaining non-A haplotypes. The mean frequency of private alleles among these haplotypes was 0.0368 with a standard deviation of 0.0236. Three private alleles in the A3 haplotypes had significant frequencies.

Population Structure

Isolation-By-Distance

Isolation-by-distance has a slightly significant ($p = 0.05$) effect on population structure across this dataset. Kernel density plots of genetic distance over geographic

distance, however, revealed more than one cluster, suggesting that forces beyond the natural clinal variation expected from dispersal limitations alone also affect population structure (**Figure 1.3**).

STRUCTURE Assignment Tests

STRUCTURE runs yielded the greatest mean L(*K*) and Δ*K* when *K* = 2 (**Figure 1.4**), suggesting that there are two populations represented in the total sample without (60%),and two of the samples were from the middle elevation group (40%). In the low *q* cluster, two of the samples had been collected from the high elevation group (20%), five of the samples were from the middle elevation group (50%), and three of the samples were from the low elevation group (30%). In the admixed cluster, two of the samples had

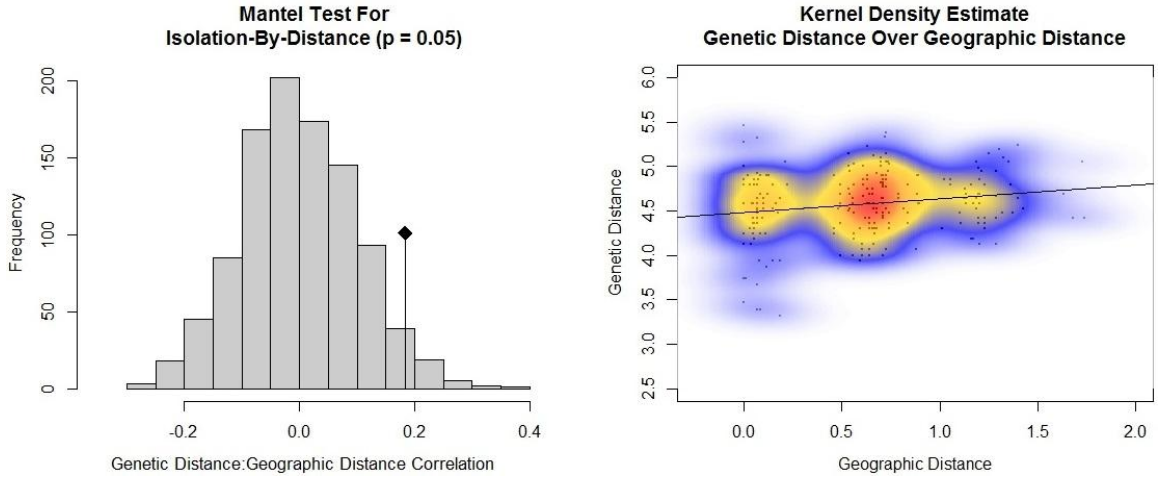

Figure 1.3: Isolation-by-distance analyses, including the output of a Mantel test (left) indicating the empirical genetic distance:geographic distance correlation calculated in the dataset (black diamond) plotted over a histogram of 999 Monte Carlo simulations run in the absence of spatial structure, and a kernel density estimate (right) of pairwise correlations from each sample showing multiple clusters. These plots indicate that there is a slightly significant (p = 0.05) effect of isolation-by-distance on the population structure here in addition to other factors affecting population structure.

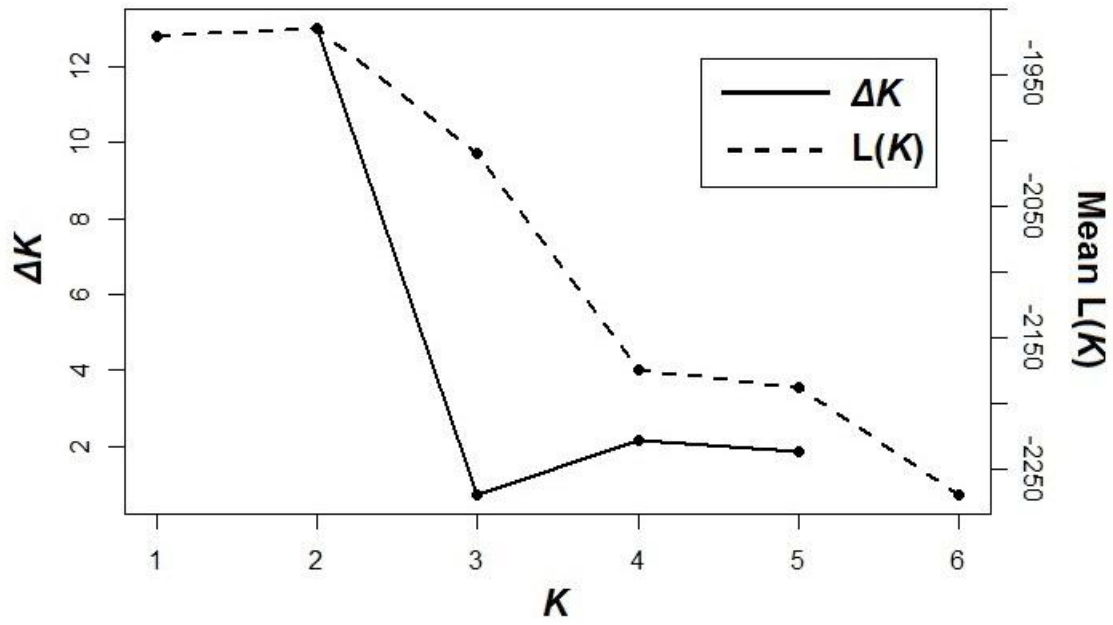

Figure 1.4: Structure Harvester (Earl and vonHoldt 2012) output showing both mean L(*K*) and Δ*K* peaking where *K* = 2, indicating that this is the most likely number of actual populations represented in the sample.

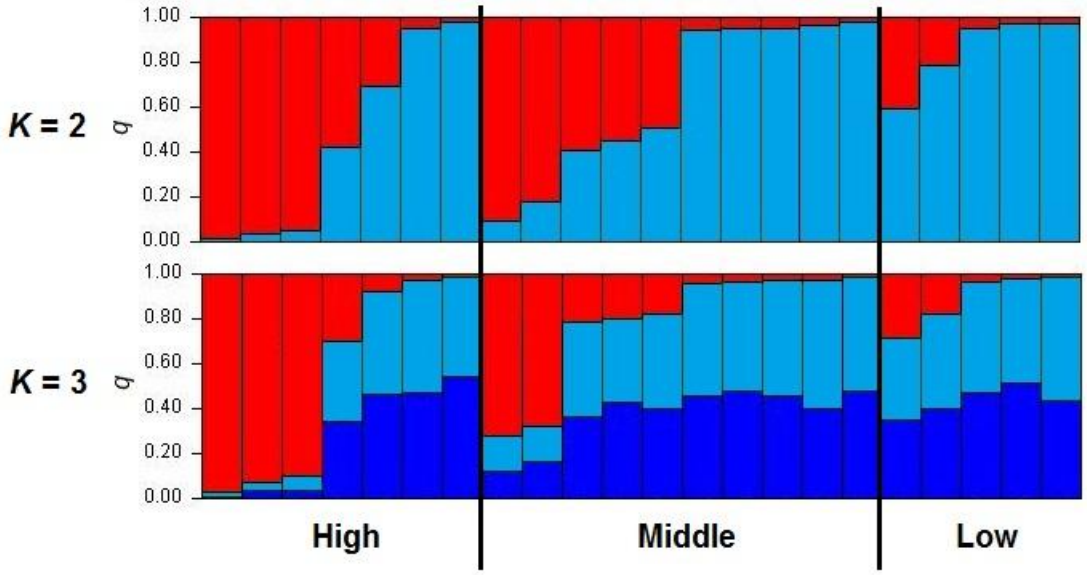

Figure 1.5: STRUCTURE bar plots for *K* = 2 (top) and *K* = 3 (bottom). Here each sample is sorted by elevation group and ordered by high *q* to low *q*, although these subdivisions were not included *a priori* in the STRUCTURE analysis. Adding the third possible cluster did not improve or change assignment test results.

been collected from the high elevation group (29%), three of the samples were from the middle elevation group (42%), and two of the samples were from the low elevation group (29%). Therefore, samples from the high and middle elevation groups had both high *q* values (maximum = 0.979) and low *q* values (minimum = 0.017), whereas *q* values in the low elevation group did not exceed 0.278.

Sex-Biased Gene Flow

Male gene flow over female gene flow (m_m/m_f) equaled 5.3015, as calculated by applying the nuclear DNA F_{ST} (0.0259) and the mtDNA F_{ST} (0.251) to Equation 7c from Hedrick and colleagues (2013). Assuming that effective population sizes are equal between the two sexes, this implies that male gene flow is over five times greater than female gene flow across the total dataset.

Geospatial and Regression Analyses

A simple linear regression model of mtDNA haplotypes over elevation shows a significant linear relationship ($p = 0.003$) with moderate goodness of fit ($R^2 = 0.3610$). *q* over elevation likewise shows a significant linear relationship ($p = 0.0382$) with moderate goodness of fit ($R^2 = 0.1977$), indicating that elevation has a significant relationship with both mtDNA and *q*. A simple linear regression model of *q* over mtDNA haplotype, however, shows an insignificant relationship ($p = 0.1786$) with poor goodness of fit ($R^2 = 0.0886$), indicating that maternal ancestry does not effect *q*.

DISCUSSION

The consistent distribution of two native Rocky Mountain mtDNA haplotypes (A and A3) across the high and middle elevation groups demonstrates that the population shares a common maternal ancestry. This in turn suggests that the population was founded naturally by foxes of Nearctic origins as opposed to either Holarctic foxes migrating through the ice-free corridor during the Wisconsin Glaciation or non-native foxes colonizing the area more recently. Phylogenically, the A haplotype is the basal haplotype of the Nearctic Clade and has a historic distribution across southern North America from the Sierra Nevada to eastern Canada. The A3 haplotype, however, is less common—it was detected in 1.3% of the 220 historic samples analyzed by Aubry and colleagues (2009), whereas the A haplotype was detected in 20% of those samples—and much more geographically restricted, being found only in the Rocky Mountains and western Canada historically. Interestingly, the A3 haplotype is derived from the O haplotype, which was historically from the Cascades and Sierra Nevada but not the Rocky Mountains or western Canada. One possible explanation for the isolation and subsequent divergence of the A3 haplotype is the reconnection of forest habitats and forest-associated populations between the Cascades and the Rockies during the Wisconsin Glaciation.

The diversity of transcontinental mtDNA haplotypes found in the low elevation group, on the other hand, suggests that this group represents the admixed population now common at lower elevations across the country. Despite its smaller sample size, this low

elevation group has greater diversity in its mtDNA than the combined high and middle elevation groups since non-native haplotypes were excluded from higher elevations.

Significant exact *G* test results support the conclusion that the combined high and middle elevation groups are different from the low elevation group. The large number of private alleles in the combined high and middle elevation groups, especially those with significant frequencies, also support the conclusion that this population has been isolated from those at lower elevations for a long time. Taken individually, the presence of private alleles with significant frequencies in the middle elevation group, but not the high elevation group, is logical since the high elevation area can not have been occupied for as long. High elevations would have remained glaciated longer following the Wisconsin Glaciation, plus it was glaciated since then during the Little Ice Age (700 to 150 years before present). Perhaps it is no coincidence that there are private alleles with significant frequencies associated with the A3 haplotype as this is the most common haplotype at middle elevations, which in turn suggests that this maternal lineage has been present in the GYE for a long time. Private alleles found at low elevations, however, are probably the result of the samples' broad distribution.

This conclusion conflicts with that of Swanson and colleagues (2005) pointing to significant differentiation between the high and middle elevation groups. One reason why we may have obtained different results is that I used a different suite of microsatellite markers that had more markers (n = 28 comparred to n = 10) and were specifically for red foxes (Moore *et al.* 2010) as opposed to generic canine markers. I also took steps to remove related individuals with ML-Relate (Kalinowski *et al.* 2006),

without which I would have obtained different results since the full sibling pair in the high elevation group was assigned to its own cluster if included in a $K=3$ STRUCTURE run. It is unclear if Swanson and colleagues likewise screened for first order relatives in their dataset, plus the useful ML-Relate tool was unavailable at the time.

Even though these results fail to support the hypothesis that high elevation foxes in the GYE originated with Holarctic foxes migrating through the ice-free corridor, the presence of a Holarctic cytochrome *b* haplotype and a novel D-loop haplotype in the low elevation group may revive this hypothesis. Given the average mutation rate of the D-loop in wild canines, acquiring the two substitutions that separate this haplotype from the nearest previously documented sequence may have taken more than 30,000 years, placing its divergence within the Wisconsin Glaciation. Plus, the open, semi-arid environment where those samples were collected is a better analog of the conditions along the glacial front and within the ice-free corridor than are subalpine forests. Foxes with intrinsic ties to boreal or subalpine forests may have indeed been excluded from such environments.

While the two samples with these haplotypes came from unrelated individuals collected about 30 km apart, more sampling and analysis of foxes living on the plains east of the GYE is warranted to examine this hypothesis. Plus the low overall sample size of the low elevation group demands greater sampling to support my conclusions for both the mtDNA and microsatellite results. I will therefore maintain contacts with trappers and snowplow drivers who have already volunteered tissue samples from roadkill foxes, as well as create new contacts with other potential sources. This may include state wildlife management agencies, area veterinary laboratories and research facilities, and regional

law enforcement patrolling roads and highways. I will also write a newspaper article about the project that highlights the need for more samples, and distribute the it to area newspapers including the Big Timber Pioneer, the Stillwater County News, the Carbon County News, the Livingston Enterprise, the Cody Enterprise, and others. My goal is to collect at least five more low elevation samples.

The population structure across the study area is affected by isolation-by-distance, reflecting the dispersal limitations of this mesopredator. This correlation between genetic distance and geographic distance, however, may itself be correlated with elevation since elevation, like geographic distance, follows a consistent gradient across the study area and between sample groups. Sampling of an additional high elevation area within the GYE but separate from the Beartooth Mountains may help clarify this discrepancy.

Even so, assignment tests demonstrated that isolation-by-distance is not the only factor affecting population structure here. The exclusion of high q values from low elevations supports the hypothesis that an elevational barrier to gene flow separates the low elevation group from those above 2,000 m. The distribution of low q values across all elevation groups in turn suggests that such a barrier is partial at best since, unlike the mtDNA barrier, low q values common at low elevations are permitted in higher elevations. The distribution of pure high q, pure low q, and admixed assignments in the high and middle elevation groups goes on to suggest that the factor(s) further affecting population structure act within elevation groups as well as between them. There was no correlation between mtDNA haplotypes and STRUCTURE assignments, indicating that

maternal ancestry does not affect the population structure observed here. Again, a stronger sample size for the low elevation group would strengthen these conclusions.

Low F_{ST} values likewise indicate that significant gene flow in the nuclear DNA occurs between the elevational groups despite the apparent barrier to gene flow affecting mtDNA. Given the low population density at high elevations, the number of migrants must be high to overcome the effect of genetic drift expected from a low number of effective breeders. Yet there is also evidence of asymmetric gene flow with respect to gender−male foxes here exhibit five times greater gene flow than females− is common in mammals and often biased towards greater male gene flow (Prugnolle and de Meeus 2004). This may reflect differences in dispersal since the median male red fox dispersal distance is seven times greater than that for females. But the ratio of male gene flow to female gene flow (m_m/m_f) calculated here is greater than that calculated for some other adjacent populations of wild canids using figures reported in other studies. This includes the eastern grey wolf (*Canis lycaon*) between Algonquin Provincial Park and the Magnetawan region in Ontario, Canada ($m_m/m_f = 0.4419$) and feral street dogs between the cities of Giza and Luxor in Egypt ($m_m/m_f = 3.1468$) (Boyko *et al.* 2009, Gewal *et al.* 2004). In contrast, it is less than the $m_m/m_f = 7.9939$ between the native Sacramento Valley red fox (*V. v. patwin*) and non-native red foxes living in the adjacent San Joaquin Valley in California using figures reported by Sacks and colleagues (2011). They also noticed stark differences in the distribution of native and non-native mtDNA haplotypes there, similar to what I observed in the GYE. The isolation of native red foxes in the GYE may therefore be similar to that of the native Sacramento Valley red fox.

A possible explanation for the asymmetry in maternal gene flow and corresponding elevational barrier I observed in the GYE may be that female reproductive cycles must be in synchrony with different climates at different elevations. Under this hypothesis, females whose reproductive cycles are predisposed to a particular climate/elevation would be limited in their ability to successfully recruit young at different elevations. To maximize survival, fox kits should be born around the time of spring green-up when weather conditions are mild and food availability is greatest. But spring green-up occurs several months earlier at low elevations in the GYE compared to high elevations. For example, the median June 1 snow depth recorded between 2011 and 2015 at the East Boulder Mine SNOTEL (1,930 m) was 0 cm, while the median snow depth at the Beartooth Lake SNOTEL (2,853 m), which is 75 km southeast of and over 900 m higher than the East Boulder Mine SNOTEL as well as 0.25 km from a known red fox den, was 160 cm. In two of those five years, the East Boulder Mine SNOTEL was snow-free by May 1 when the median depth at Beartooth Lake is over 200 cm, and on one of those years it was snow-free by April 1 when the median snow depth at Beartooth Lake is over 190 cm. So while the snowpack is rapidly melting between April and May at 1,930 m in the GYE, it is still accumulating at 2,853 m. Kits emerging from their den in mid-May at lower elevations, therefore, would encounter ideal conditions, whereas kits emerging in mid-May at higher elevations would encounter conditions resembling, or even more severe than, mid-winter conditions at lower elevations. And if the kits can not survive in that environment, neither will their parents' DNA.

Anecdotal observations of fox dens in the GYE support the hypothesis that

parturition occurs later at higher elevations. Three fox kits observed in mid-May at a den on the East Gallatin River (1,320 m) near Bozeman, Montana, were almost indistinguishable in size and color from the adult fox at the den. Fox kits observed in early June at dens on Colter Pass (2,450 m) and Lulu Pass (2,770 m) near Cooke City, Montana, in contrast, were substantially smaller than adults and lacked adult coloration (Dwain Hackman, personal communication). This suggests that the kits near Bozeman were born earlier than those near Cooke City. Delayed parturition at higher elevations compared to lower elevations has also been observed in other mammals including bison, bighorn sheep (*Ovis canadensis*) and the New Mexico meadow jumping mouse (*Zapus hudsonius luteus*) as well as in other red fox populations (Frey 2015, Hill 2007, Naughton 2012, Whiting 2008).

Yet the question remains as to what mechanisms may be facilitating delayed parturition at higher elevations. Possible explanations include delayed timing of estrus and breeding at higher elevations compared to lower elevations and/or a prolonged gestation period at higher elevations compared to lower elevations. These hypotheses may be tested by observation of pair bonding and scent marking characteristic of breeding behavior through non-invasive snow tracking over an elevational gradient. Further observation of kit development at different elevations is also warranted.

It is also unclear whether differences in the timing of reproductive cycles is truly an adaptive response with a heritable genetic basis or the product of individual plasticity for which canines are reknowned. This could likewise be true for other characteristics of montane foxes, such as novel coat colors, hairy feet, and forest habitat selection behavior,

that distinguish them from foxes at low elevations. One way to examine this would be a "common garden" experiment (Matesanz *et al.* 2012, Whitehead *et al.* 2011) in which individual foxes born at lower elevations and displaying characteristics of low elevation foxes are transplanted into high elevations and monitored over multiple seasons to see if they start displaying montane fox characteristics. While this experiment would be difficult in the wild, some conditions like temperature and snow can be replicated in a laboratory, therefore some responses such as the timing of reproductive cycles and the length of hair on the feet could be tested at a captive research facility. Also, the preponderance of light coat colors at high elevations–all nine of the foxes sampled at high elevations displayed the light "cross" color phase, whereas the majority of fox records assembled by Fuhrmann (1998) at lower elevations displayed the common "red" color phase–and the presence of F_{ST} outlier loci on chromosomes with genes controling hair characteristics are evidence that coat color there may indeed be an adaptive response.

High elevations in the GYE serve as a refugium of native red fox genetics. This lineage has persisted in the ecosystem since the Pleistocene despite subsequent environmental changes and anthropogenic modification of the landscape. The existence of this lineage speaks to the complexity of population differentiation within what ostensibly appears to be the continuous distribution of a widespread species, while the existence of this refugium demonstrates the continued ecological integrity of the GYE.

CHAPTER 2

FOXES AND FORESTS: EXAMINING HABITAT SELECTION OF RED FOX IN THE GREATER YELLOWSTONE ECOSYSTEM THROUGH TELEMETRY, SNOWTRACKING, AND FECAL CONTENT ANALYSES

INTRODUCTION

Geographically isolated and genetically distinct montane red fox (*Vulpes vulpes*) populations across the western United States share a habitat preference for subalpine forests (Aubry 1983, Aubry *et al.* 2009, Crabtree 1997, Fuhrmann 1998, Perrine *et al.* 2007, Sacks *et al.* 2010, Sacks *et al.* 2011, Statham *et al.* 2012, Van Etten 2006, Volkmann *et al*. 2015). Although surprising for a species that is generally a predator of small mammals in more open habitats, subalpine forests may simulate the boreal forests of ancestral populations, facilitate a means of avoiding competition with larger carnivores like coyotes (*Canis latrans*), and/or provide additional niches for the highly adaptable red fox−the most widely distributed terrestrial mammal in the world (Aubry 1983, Aubry *et al.* 2009, Crabtree 1997, Fuhrmann 1998, Hartová-Nentvichováa *et al.* 2010, Perrine *et al.* 2007, Sacks *et al*. 2010, Sacks *et al.* 2011, Statham *et al.* 2012, Van Etten 2006, Volkmann *et al*. 2015). Given the ecological limitations of these environments and their fragmented distribution across the isolated Sierra Nevada, Cascade Range, and Rocky Mountains, selection for subalpine forests may thus effect an ecological barrier between historic native populations as well as the admixed population now common at lower elevations across North America (Aubry *et al.* 2009, Kamler and Ballard 2002, Sacks *et*

al. 2010, Sacks *et al*. 2011, Statham *et al.* 2012, Volkmann *et al* . 2015). This widespread and expanding admixed population comprises lineages from across the fox's circumboreal range united by natural expansion, anthropogenic land use changes, and/or translocations (Aubry *et al*. 2009, Kamler and Ballard 2002, Statham *et al.* 2012, Volkmann *et al*. 2015). Forest habitat selection may therefore be a behavioral mechanism preserving native fox genetics within their historic distribution.

After observing forest habitat selection by red foxes of the Cascade Range, Aubry (1983) theorized that these endemic populations may be relics of a boreal population that was more widespread during the Pleistocene. He suggested that the southward expansion of boreal forests at the onset of recent ice ages facilitated the expansion of boreal taxa, including foxes, while the subsequent retraction of these habitats to higher latitudes and elevations at the end of the ice age led to habitat fragmentation, isolation, and population differentiation. Similar patterns of evolutionary divergence caused by climatic and biogeographic changes have been observed in other nearctic taxa like the red-backed vole (*Cletheronymys gapperi*) as well as neotropical taxa like rainforest caddisflies (Trichoptera) (Cook *et al.* 2004, Múrria and Hughes 2008). Indeed, it was in his study of neotropical forest birds whose ancestors had been isolated by Pleistocene grassland expansions that Haffer (1969) introduced the "speciation pump" concept, suggesting that modern lineages were shaped by periodic isolation and divergence as well as connectivity and gene flow associated with long-term cyclical processes like glaciation.

Forest habitat selection has since been observed in other montane red fox populations. The Sierra Nevada red fox, which uses a variety of high elevation habitats

including subalpine forests in the summer, descends to mature conifer forests in the winter even though these habitats occupy less than 7% of its available range (Perrine *et al.* 2005). Foxes at high elevations in the Rocky Mountains display an increased frequency of light coat colors corresponding with increasing selection of subalpine forest habitats, suggesting an evolutionary connection to this behavior (Crabtree 1997, Fuhrmann 1998, Swanson *et al.* 2005). These montane populations have long been noted for their distinctive morphologies and are currently classified as mountain range-specific subspecies: *cascadensis* in the Northern Cascades, *necator* in the Sierra Nevada and Southern Cascades, and *macroura* in the Rocky Mountains (Churcher 1957, Kamler and Ballard 2002, Statham *et al.* 2012b). From the Greek *makros* for "long" and *oura* for "tail," *macroura* gives precedence to the name "great-tailed fox" as the Rocky Mountain fox was once called, described by Baird (1852) as "at once distinguished by the great length of the tail, which exceeds that of the [eastern red fox] species by six inches, and more." Subsequent phylogeographic analyses examining the spatial distribution of gene sequences and the time required for new sequences to arise confirm that the origin and ultimate differentiation of North American foxes corresponds with the timing of Pleistocene glaciation (Aubry *et al.* 2009).

The red fox is also a generalist species known for its diverse diet and ability to occupy a variety of habitats (Aubry *et al.* 2009, Hartová-Nentvichováa *et al.* 2010, Kamler and Ballard 2002). This habitat plasticity is one reason why European foxes introduced to Australia became such a successful invasive species (Newsome *et al.* 2014, Robley *et al.* 2014). The recently described Sacramento Valley red fox (*Vulpes vulpes*

patwin) exemplifies habitat plasticity as this endemic population is genetically related to the Sierra Nevada subspecies *necator* yet inhabits an arid, open, low elevation habitat: a montane red fox in a non-montane environment (Sacks *et al.* 2010). Such adaptability in turn suggests an inherent capacity to colonize novel or marginal environments. So an alternative hypothesis explaining the selection of forest habitats by montane red fox populations is that they are avoiding competition with coyotes for resources and territory.

Even though Van Etten and colleagues (2007) observed significant forest habitat selection by red foxes in the Lamar Valley of Yellowstone National Park, this selection pattern relaxed somewhat during the evening when coyotes were less active and the winter when coyotes were less mobile in deep snow. This led them to suggest that some of the forest habitat use they observed may be driven by avoidance mechanisms in response to competition. Red foxes with sympatric coyote populations at low elevations across the midwestern and eastern United States avoid competition by relocating to marginal or fringe habitats (Harrison *et al* 1989, Klett 1987, Levi and Wilmers 2012, Sargeant and Allen 1989, Sargeant *et al*. 1987, Voight and Earle 1983). Such caution is warranted as coyotes sometimes kill red foxes (Sargeant and Allen 1989). Arctic foxes (*Vulpes lagopus*) show a similar response to the northward expansion of larger red foxes– sterilized red foxes have even been proposed as a biocontrol measure on Aleutian Islands where introduced arctic foxes threaten native seabird populations (Frafjord et al. 1989, Gallant et al. 2012, Pamperin et al. 2006, Schmidt 1985, Tannerfeldt et al. 2002).

The fox population on the Beartooth Plateau of the Greater Yellowstone Ecosystem (GYE), which is adjacent to and higher in elevation than Van Etten's Lamar

Valley study area, is free of competition with resident coyotes (Crabtree and Sheldon 1999). Although transient coyotes are occasionally observed there in the summer, deep snow excludes them in the winter. Red foxes, on the other hand, live and breed there year round at elevations as high as 3,300 m, making this the highest known fox population in North America (Crabtree 1993, Crabtree and Sheldon 1999, Swanson *et al.* 2005, Statham *et al*. 2012b). The disparity in oversnow mobility is due to the coyotes' lesser foot size-to-body weight ratio: in the Lamar Valley, the mean weight of coyotes is three times greater than that of foxes, yet their mean foot size is less than three times that of foxes (Fuhrmann 1998). This results in increased foot loading, track sink, and energy expenditure for coyotes moving through snow. Plus, foxes at high elevations have thick fur covering the entire foot including the toe and heel pads, further increasing the foot's surface area and the snowshoe-like effect of decreased foot loading. Canada lynx (*Lynx canadensis*) have a similar snow-adapted morphology, and Fuhrmann (1998) recorded foot loading values in the foxes he measured comparable to those of lynx.

Therefore, the primary goal of this study was to assess selection of forest habitats by a presumably native red fox population in an environment free of competition with coyotes. I predicted that significant selection of forested habitats over non-forested habitats would support the hypotheses that selection of forest habitats by this and other montane fox populations is associated with the boreal forests of ancestral populations (Aubry 1983, Fuhrmann 1998, Perrine *et al.* 2005). Insignificant selection or use of forest habitats less than that expected from availability, however, would support Van

Etten and colleagues' (2007) hypothesis that competition with coyotes drives increased forest habitat use by foxes at lower elevations in the GYE. Specific objectives included:

1. Acquiring habitat use data from the high elevation Beartooth Plateau through telemetry and snowtracking, and exploring it with Resource Selection Probability Function (RSPF) models using influential environmental covariates (Lele and Keim 2006, Manlove *et al.* 2011).
2. Assessing non-random selection of forested, non-forested, and edge habitats (K = 3) as well as more refined (K = 6) land cover categories with the Euclidean distance method (Conner and Plowman 2001) used by Van Etten and colleagues (2007) in the nearby but lower elevation Lamar Valley.
3. Examining interannual variance in the use of specific habitat and food resources by analyzing forest cover types (Despain 1990) recorded and fox scats collected while snowtracking over two winters (2013 and 2014).

The first objective assessed the ability of the response data collected to represent the red fox population on the Beartooth Plateau as well as identified landscape features and quantitative ecological thresholds affecting resource selection. The second objective assessed habitat selection with methods similar to those Van Etten and colleagues (2007) used in the Lamar Valley, providing a means of comparing selection in two populations differing with respect to competition with coyotes. The final objective identified specific resources influencing red fox habitat selection at high elevations while it also tested the stability of selection patterns observed in the face of varying environmental conditions.

Even though the study's two year time frame limits my ability to assess long-term

trends, I was fortunate to capture a cyclical environmental event that effects a significant ecological response, providing uncontrolled, quasi-experimental conditions to test variance in resource selection. Whitebark pine (*Pinus albicaulis*) cone production was high the summer before 2013, but low the next summer (Haroldson and Podruzny 2013, Haroldson 2014). Snowfall was also substantially less in 2013 than it was in 2014, affecting access to habitats and resources (**Figure 2.1**).

The results show that even though there is strong selection for forest habitats in this population, significant selection of edge habitats–almost 73% of response data was recorded within 30 m of the forest edge–counteracts any differences in the selection of forested over non-forested habitats. This suggests that overall resource scarcity in alpine

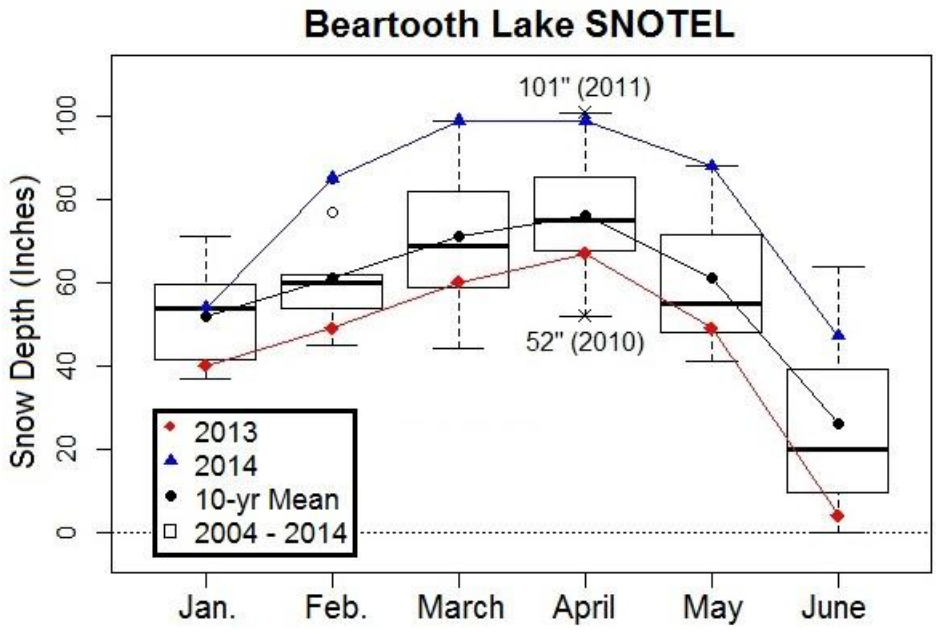

Figure 2.1: Interannual variance in monthly snow depth (inches) reported at the Beartooth Lake SNOTEL for 2013, 2014, and the ten year average, including maximum and minimum values and years for peak snowpack (April 1).

environments and greater resource availability in heterogeneous edge habitats may influence red fox habitat selection there as much as or more than intrinsic habitat preferences and competitive pressures do at lower elevations. Forest habitats and the resources therein nevertheless play a critical role in the life histories of these montane red foxes and the persistence of their native populations. The significant effect of whitebark pine on the foxes I observed–the first time, to my knowledge, that this mesopredator has been reported using the food resource–helps explain how the species survives in such an extreme environment. It also expands the known ecological role and importance of the whitebark pine, recognized as a keystone species and, like the Sierra Nevada red fox population, one for which U.S. Endangered Species Act protection is warranted (USFWS 2011, USFWS 2015). Conservation of native montane red fox populations, therefore, is tied to the conservation of alpine biodiversity.

MATERIALS AND METHODS

Study Area

Spatial use data was collected from a roughly 260 km^2 area covered by telemetry and snowtracking centered at Beartooth Lake (44.9446ºN, 109.5890ºW) in the Shoshone National Forest, Wyoming, USA. About 58.74% of the land area is forested and 41.26% is non-forested (GAP, USGS). It is characterized by an elevational habitat gradient ranging from sagebrush montane foothills at low elevations (2,000 m - 2,300 m) to montane lodgepole pine and douglas fir forests at middle elevations (2,300 m - 2,600 m), subalpine spruce-fir and whitebark pine forests with mesic and xeric meadows at higher elevations (2,600 m - 2,900 m), and alpine tundra and rocks up to 3,400 m. I focused on elevations above 2,000 m since resident coyotes are rare above this threshold. It also corresponds with the elevation where the frequency of lighter red fox coat colors significantly increases (Swanson *et al.* 2005).

Data Collection

Radio Collaring and Telemetry

Foxes were live trapped using methods described in the previous chapter (please see "Trapping and Sample Collection" on page 8). Juvenile foxes were fitted with VHF collars (Advanced Telemetry Systems, Isanti, MN) to monitor survival and dispersal, while resident adult foxes expected to remain in the study area were fitted with GPS collars (Telemetry Solutions, Concord, CA). The GPS collars were programmed on a

three tier fix schedule at increasingly finer resolutions for 2nd order (home range), 3rd order (internal anatomy of home range), and 4th order (movement path) selection analyses (Johnson 1980, Van Etten *et al.* 2007). Battery life was anticipated to accommodate a year of data collection. Each collar schedule therefore included:

1. Two relocation attempts in the morning and evening every day for the 2nd order analysis, totaling 240 independent relocations attempts per collar per season.
2. Hourly relocation attempts one day every week for the 3rd order analysis, totaling 336 relocation attempts per collar per season.
3. Relocation attempts every 15 minutes for two day bursts every month for the 4th order analysis, totaling 768 relocation attempts per collar per season. This sample was supplemented with snowtracking data.

GPS collars also had a VHF transmitter, internal memory card, timed drop-off mechanism, and UHF remote download capability to retrieve the data. Both the GPS and VHF collars had mortality sensors that increased the VHF transmission interval when the collar was inactive for more than 12 hours. VHF signals were used to monitor collars and collect additional relocations by triangulation estimated with Locate III (Nams 2006).

Eight foxes were collared during three trapping seasons, which is a strong sample size here given the extensive trapping effort and the low population density in this extreme environment (**Table 2.1**). An adult male (M343) captured in June 2012 and fitted with a VHF collar was recaptured in March 2013 and refitted with a GPS collar (thereafter M800). Another adult male (M000) was captured in the final season of the study, so his GPS collar was reprogrammed with a more intensive sampling schedule to

ID	Date	Min. Temp. (°C)	Days Since Snow	Trap	Age	Kg	Collar	Re/At: VHF GPS	Fate
M343	6/3 2012	3.4	8	Box	3 - 4	5	VHF	49/55 NA	Replaced* 3/4/13
F100	3/2 2013	-4.8	8	Log Cabin	> 6	NA	GPS	1/122 0/0	Unknown
F363	3/3 2013	-3.2	9	Log Cabin	1 - 2	3.9	VHF	34/104 NA	Coyote 5/14
M800*	3/4 2013	-15.7	< 1	Log Cabin	4 - 5	5.9	GPS	10/117 1.3k/1	Unknown
F324	3/5 2013	-17.7	1	Log Cabin	2 - 3	4.1	VHF	6/31 NA	Wolf 4/13
F700	3/12 2013	-10.6	< 1	Log Cabin	2 - 3	4.8	GPS	5/125 0/0	Alive 2/24/15
M500	3/13 2013	-3.0	1	Log Cabin	2 - 3	4.1	GPS	2/107 0/0	Unknown
F306	1/27 2014	-14.3	14	Log Cabin	1 - 2	4.5	VHF	13/62 NA	Unknown
M000	2/3 2014	-18.2	4	Log Cabin	3 - 5	5	GPS	7/49 0/0	Human 5/14

Table 2.1: Red fox capture data, tracking success (relocations/attempts for VHF triangulations (including live visuals) and GPS remote data downloads), and fate from nine collars on the Beartooth Plateau, Shoshone National Forest, Wyoming, USA. Data on minimum temperature the night of capture and days since last snowfall−conditions that may influence trapping success−were obtained from the Beartooth Lake SNOTEL. M343 was recaptured on 3/4/2013, and his collar was replaced with a GPS collar (M800).

take advantage of battery life. Three collared foxes died and were recovered during the study: F324 was killed by wolves near a bull elk carcass in April 2013, F363 was killed and eaten by coyotes in May 2014, and M000 was killed near the highway in May 2014.

Resulting GPS relocations totaled 200 2nd order, 480 3rd order, and 576 4th order relocations collected from one individual (M800) via remote download during the late winter season 2013. No additional data was retrieved from this or the other four GPS collars. M800 went missing in mid-July 2013 one month after the remote download. F100, M500, and F700 all went missing within two weeks of being deployed despite over

100 search attempts including an aerial search with an experience telemetry pilot in May 2014 (Doug Chapman, Bozeman, MT). One individual with a non-functioning GPS collar, most likely F700, has been seen near Cooke City as recently as February 2015 (Tom Wolfe, personal communication). And even though M000's collar was retrieved– the drop-off mechanism failed, but the collar had been cut off with a knife and dropped on the side of the road–it failed to collect any data.

Snowtracking

I followed fox tracks in the snow to supplement and validate habitat use data from the GPS collars as well as collect evidence of behavior that collars can not observe, including scent marking on territorial boundaries, foraging locations, and sometimes even foraging success. Eight transects, averaging 4 km in length, spaced 2 km to 4 km apart on alternating sides of a 12 km stretch of the Beartooth Highway (U.S. 212), and following an established road or trail for repeatability, were skied regularly between January and May 2013 and between January and April 2014. The circuit of transects was skied in a randomized order, and prioritization of fox tracks encountered alternated between first-to-last and last-to-first with each cycle. Fox tracks crossing the transect were backtracked, and the locations of fox activity sites (ie. resting, scentmarking, or foraging behavior) were recorded as "activity point" waypoints on a handheld GPS along with the following site attributes: snow depth, snow type, track sink, track number, track gait, slope and aspect, and distance to edge. One especially important site attribute recorded was the forest cover type describing the plant community through its dominant tree species and current successional stage, defined specifically for the GYE (Despain

1990) and used in previous mountain fox studies there (Fuhrmann 1998, Van Etten *et al.* 2007). When the tracks could no longer be followed, they were retraced while GPS path data was recorded on 1 m intervals and additional "habitat point" waypoints were recorded every 15 minutes along with site attribute data. These habitat points, plus the first and last waypoint recorded on each track, were used to supplement GPS data collected on the same interval. This temporal habitat point sampling frame differs from that of Fuhrmann (1998) and Van Etten and (2006), who collected habitat points on a 250 m spatial sampling frame; otherwise I used similar methods for compatibility and comparability with their data. If time allowed, I repeated this with the next set of tracks.

The 4 km transect interval used here is based on Hersteinsson and Macdonald's (1982) home range analyses and replicates Fuhrmann's (1998) methods, however actual individual home ranges in this alpine environment are probably much larger than this sampling frame is designed for. Van Etten and colleagues (2007) reported an average year round home range of 9.73 km^2 for male and female foxes in the Lamar Valley. Relocations from a male fox (M343/800) displaying den provisioning behavior in this study area showed that he used a 15 km^2 area that summer (2012), then covered an 87 km^2 area the following spring in a 90% minimum convex polygon (MCP) calculated in R with the "adehabitat" package (Calenge 2006). Substantial dispersal and extra-territorial movement distances were also documented in other individuals: an adult female (F700) was captured more than 30 km away from her usual territory, a subadult female (F363) was documented over a 90 km^2 square mile area in the two years she was tracked, and a genetic sample was collected from an adult male (L1"Sunlight") over 20 km south of and

across a significant canyon from his mother (F100). For perspective, Lucherini and Lovari (1997) reported home ranges between 0.06 km^2 and 32 km^2 in their review across the species' global distribution. Yet such large areas and distances covered by individuals here, and most importantly their substantial overlap, supports the ability of the sampling frame to collect data from multiple independent individuals.

In 2013, 50 transects were skied (~125 km), 12 sets of fox tracks were tracked for ~40 km, and 78 habitat points and 151 activity points were recorded with site attributes. In 2014, 76 transects were skied (~190 km), 17 sets of fox tracks were tracked for ~70 km, and 137 habitat points and 202 activity points were recorded with site attributes. The majority of this data (73%) was collected within 30 m of the forest edge (Figure 2.2)

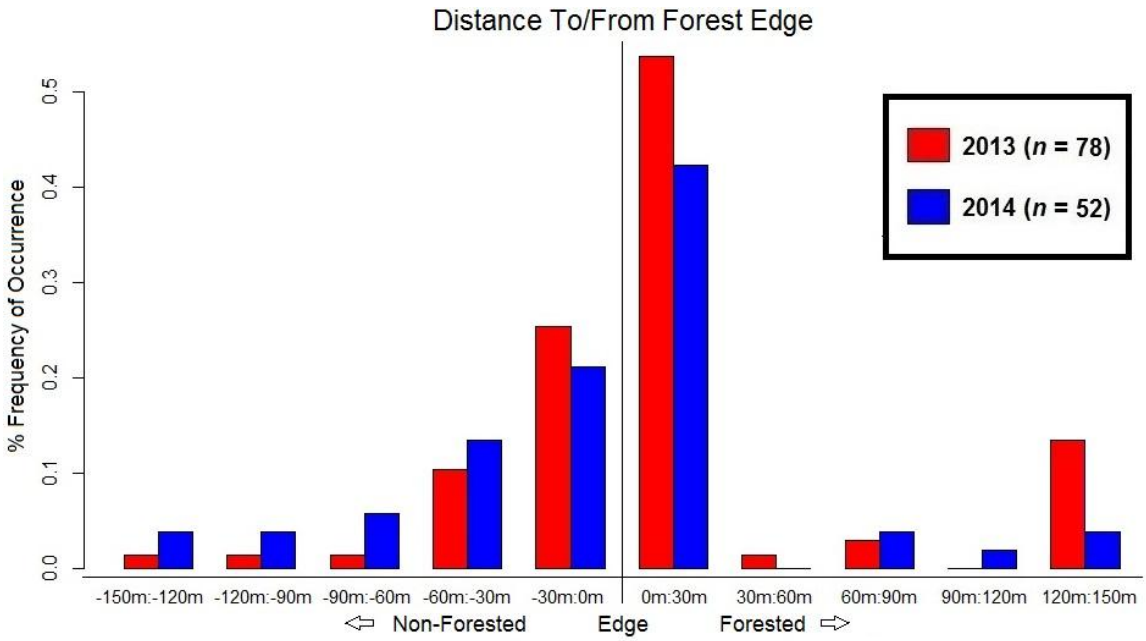

Figure 2.2: Percent frequency of occurrence of distance to edge estimates recorded with snowtracking data showing a preponderance of edge use and overall similarities in the 2013 (red) and 2014 (blue) distributions. Yet 2013 had more forest habitat use > 120 m from the edge while 2014 had more non-forested habitat use > 120 m from the edge.

Fecal Contents

For additional insight on the food habits driving habitat selection, fox scats were collected throughout the study area while snow tracking, at trap sites, and opportunistically during travel. Scats were stored frozen and later baked at 60˚C for 24 hours to kill zoonotic parasites, then dissolved in water and rinsed through wire mesh screens to separate undigested content for identification (Kelley and Garton 1997; Fortin *et al.* 2012). Taxonomic keys (Foresman 2001; Moore *et al.* 1974) and museum specimens (University of Montana Phillip L. Wright Zoological Museum) were used to identify teeth, jaws, bones, hair, paws, claws, and other material to species when possible. Additional identification help was provided by Paul Hendrix at the Phillip L. Wright Zoological Museum. The proportion of each species in each scat, along with the date and location of collection, were used to create pie charts identifying samples that most likely came from the same individual so that they could be removed as duplicates. These proportions were also used to establish a minimum threshold to exclude indigestible content persisting in low quantities, such as elk hair, that would obscure food items of greater digestibility but lesser quantity, such as voles. That way I could focus on the food items the foxes were focusing on.

In 2013, 30 fox scats were collected, two of which were subsequently removed since they appeared to be duplicates from the same individual. In 2014, 39 fox scats were collected, six of which were subsequently removed since they appeared to be duplicates.

Data Analysis

Resource Selection Probability Function (RSPF) Models

I first assessed the data collected for its ability to represent the population using RSPF models in the Ecosystem Assessment Geospatial Analysis & Landscape Evaluation System (EAGLES) (Manlove *et al*. 2011). These ArcGIS- (ESRI, Redlands, CA) based tools provide means of statistically and visually examining the spatial relationships between response data and influential environmental covariates. Inputs include response-derived "use" and "available" spatial data–generally a 1:5 ratio–and covariate raster data, and the primary outputs are spatially explicit estimates of the probability of resource selection (RSPF) visualized in GIS with a probabilistic response surface.

"Use" data was subset by the year (2013 versus 2014) and method (telemetry versus snowtracking) of collection. That way subsets could be modeled individually and in combinations to assess the effects of sample size and variable environmental conditions on model outcome as well as the overall performance of individual models. For telemetry inputs, the 15 minute (4th order) GPS relocations were used. To avoid clusters of GPS relocations from inactivity that would bias RSPF analyses towards rest sites, only one location per 50 m per day was allowed. For snowtracking inputs, the first, last, and 15 minute "habitat points" were used. Use subsets thus included:

1. 2013 snowtracking data alone (n = 92),
2. 2014 snowtracking data alone excluding two transects (Muddy Creek and Ghost Creek) that were not sampled in 2013 (n = 54),
3. 2013 and 2014 snowtracking data excluding the two transects (n = 146),

4. 2013 and 2014 snowtracking data with all transects (*n* = 238),
5. GPS collar data excluding the month of May when snowtracking data was not collected (*n* = 110),
6. a combination of GPS collar data (excluding May) and 2013 snow tracking data since that was the only year that GPS collar data was collected (*n* = 202),
7. a combination of GPS collar data (excluding May) and snow tracking data from 2013 and 2014 excluding the Muddy Creek and Ghost Creek transects (*n* = 256),
8. a combination of GPS collar data (excluding May) and snow tracking data from 2013 and 2014 with all transects (*n* = 348), and
9. a combination of all GPS collar data and all snow tracking data (*n* = 382).

"Available" data was defined for each respective use subset. Locations were generated randomly, numbered five times that of the respective use locations (Manlove *et al.* 2011), and were bound within an MCP containing the use locations surrounded by a 1 km buffer.

Covariate data was assembled in a three step approach: variables thought to affect species response were first *identified* in a conceptual narrative model (**Figure 2.3**). Of particular interest was how land cover affects red fox habitat selection. Raster data was then *acquired* from public-access sources including the Custom Online Aggregation & Summarization Tool for Environmental Rasters (COASTER) (Yellowstone Ecological Research Center; Bozeman, MT), the Snow Data Assimilation System (SnoDAS) (National Snow and Ice Data Center; Boulder, CO), and the U.S. Geological Survey's National Land Cover Dataset (NLCD) and Gap Analysis Program (GAP).

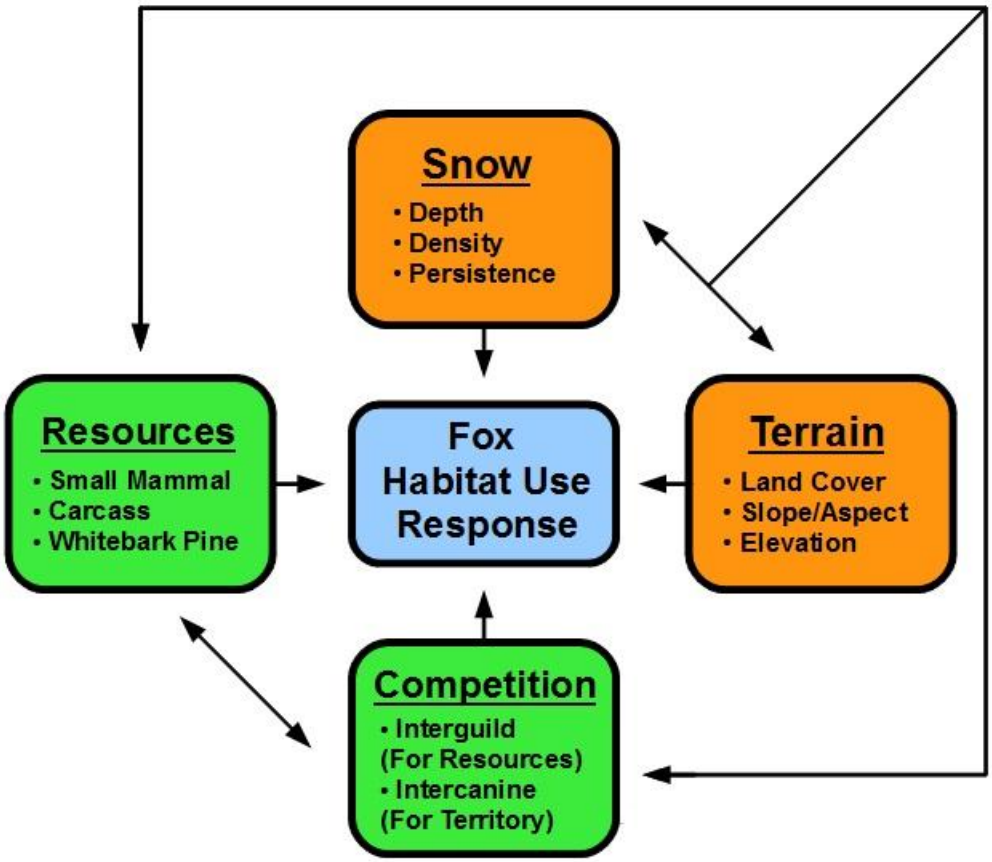

Figure 2.3: Conceptual narrative model guiding the development of an RSPF model predicting red fox resource selection at high elevations. Biotic (green) and abiotic (brown) factors expected to affect species response are listed, while arrows between factors indicate possible interactions and correlations between candidate covariates.

I used a 500 m resolution mean annual net primary production (NPP) product from the Carnegie-Ames-Stanford Approach (CASA) model downloaded from COASTER. This covariate primarily served as a surrogate for land cover since the GLM component of the RSPF model handles continuous data, like NPP, better than categorical data, like land cover classifications. I can justify this since categorical land cover classifications identified by average NPP would naturally sort from least to most

productive across a continuous gradient of ascending NPP. CASA NPP estimates (g C m^{-2}/year) range from 0 to 20.5 in this study area, and summary figures from NPP values extracted at snowtracking points with non-forested land covers (minimum = 6.91, maximum = 17.82, mean = 14.29, sd = 1.96) are lower than those extracted from points with forested land covers (minimum = 11.65, maximum = 20.25, mean = 15.38, sd = 1.86). Side-by-side visual comparisons of CASA NPP estimates and independent GAP land cover classifications also show similarities in the distributions of high NPP and forests and of low NPP and open areas (**Figure 2.4**). This suggests that NPP can be a proxy for broad landscape classifications such as forested and non-forested, while it may also reflect more subtle differences within these broad classifications affecting species response, prey habitats, and other covariates. CASA estimates NPP using spaceborne measurements of photosynthetically active radiation, temperature, and precipitation. I also included 1 km average snow depth estimates for 2013, 2014, and the two years combined from SnoDAS, which uses SNOTEL data and temperature- and precipitation-based numeric weather prediction models to recreate past snowpack characteristics. Slope, aspect, and elevation data were drawn from 30 m Digital Elevation Models (DEMs; NLCD) using the Spatial Analyst tools in ArcGIS.

Acquired rasters were then *processed* in ArcGIS for geospatial consistency. Coordinate systems were set to Universal Transvere Mercator (UTM) 1983 North American Datum (NAD 83) Zone 12, cell sizes were scaled to 30 m, and spatial extents were clipped to uniform bounds, in that order, to ensure proper spatial alignment when values from each layered raster are extracted to each cell in a merged data array.

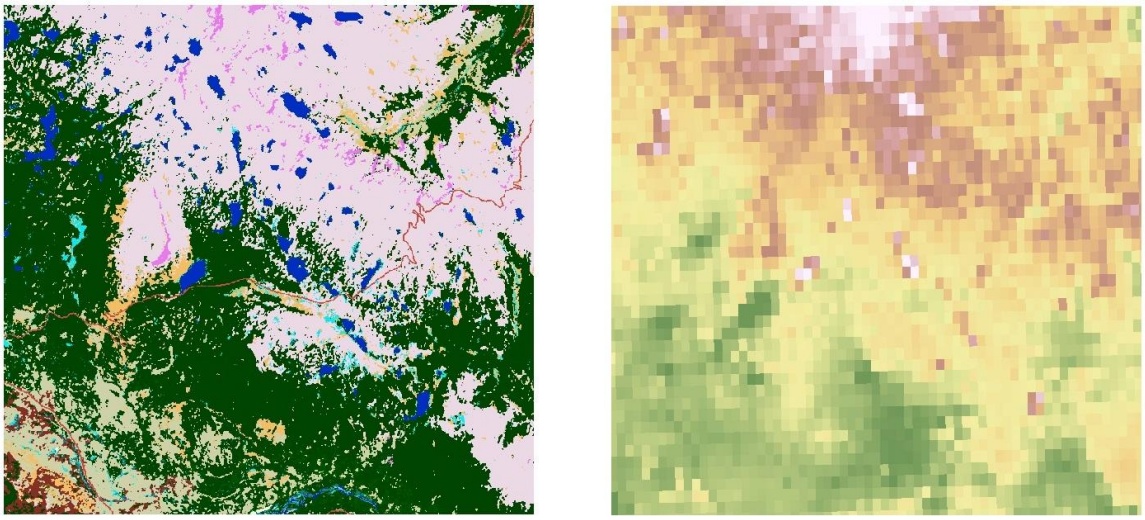

Figure 2.4: GAP imagery-based land cover classifications (left) and CASA sensor- and model-based NPP estimates (right) used as a surrogate for land cover in the RSPF model. The images show similarities in the geospatial distribution of NPP values (g C m^{-2}/year) and land cover classifications that justify using NPP to represent land cover.

Having assembled all of the inputs, I performed a series of RSPF runs with the nine use subsets and covariate rasters producing significant and interpretable effects. Component univariate general linear models are first fit to the use data. EAGLES provides the resulting plots and p values in a graphic interface allowing users to select the covariates, polynomial terms, and link function to apply in the final RSPF model–for these runs, all possible covariates, both first and second order polynomial terms for each covariate, and a logit link function were selected. RSPF uses weighted distributions when comparing use and available data so that the available points do not have to be unused points, which is a limiting assumption made in similar species distribution models (Lele and Keim 2006).

The final output is a probabilistic response surface: a spatially-referenced raster of the probabilities (RSPF values) grid cells will be selected given the input covariates.

EAGLES also provides significance figures (p values), log likelihood estimates, and goodness of fit estimates (Hosmer-Lemeshow) in a model fit summary. The Hosmer-Lemeshow goodness of fit estimate is calculated from observed:expected ratios drawn from an additional set of universal random locations covering the entire spatial extent of the covariate rasters, not just the MCP defining the available area, and numbering many times more than the number of available points. Since the test statistic it uses follows a chi-squared distribution, the p value describes the probability that results will land within the righthand tail of the distribution, thus a low p values suggests a poor model fit (Hosmer and Lemeshow 1980).

I visually compared the resulting probabilistic response surfaces from each run to identify differences and similarities between outputs and to assess their biological reasonability. I also examined statistical measures of model fit to identify input datasets that produced robust results in spite of sampling challenges . The best model with the most parsimonious use of significant covariate terms fit to the most robust response data was identified as the final RSPF model best representing actual red fox resource selection. I used its probabilistic response surface and univariate GLM output to identify landscape features and quantitative ecological thresholds affecting RSPF values.

Euclidean Distance Method of Assessing Non-Random Selection

Habitat selection was assessed for $K = 3$ land cover classifications (forested, non-forested, and edge) as well as $K = 6$ classifications (spruce-fir (SF), lodgepole pine-douglas fir (LPDF), xeric meadows and shrublands (Xeric), mesic meadows and shrublands (Mesic), sagebrush meadows (Sage), and rocks). GAP 30 m land cover data

derived from satellite imagery were classified and clipped to the spatial extent of an MCP containing all possible use inputs, then new 30 m "Euclidean Distance" rasters were generated for each classification using the Spatial Analyst package in ArcGIS. The distance (m) from each land cover classification was extracted to every input use point and averaged; this was likewise done with 181,000 random points saturating the available MCP to define the mean expected distance.

For each land cover classification, the quotient of mean observed distances over the expected mean distance is subtracted by one to arrive at Conner and Plowman's (2001) mean distance ratio. With this ratio, negative values indicate strong selection or use that is greater than expected from availability. This analysis was done for each set of use inputs. I also performed chi-squared tests in the $K = 3$ analysis to assess the significance in selection of forested versus non-forested habitats and of edge versus non-edge habitats.

Finally, I estimated Type I (false positive) and Type II (false negative) error rates in the GAP land cover data by validating it with cover type observations (Despain 1990) recorded while snowtracking. I also subset the validation data within and beyond 30 m of the forest edge to examine the consequential interaction of substantial (73%) edge habitat use in the response data and the 30 m resolution of remote sensing land cover data.

Interannual Variance in Habitat Use and Food Habits

Counts of cover types recorded while snowtracking were summed in contingency tables for each year, and a Fisher's exact test implemented in the R Statistical Computing Platform (R Core Team 2013) was used to compare contingency tables for significant

variance. This test of independence was chosen over the similar Pearson's chi-squared test as it does not assume a large sample size and can accommodate categories for which counts are low or even zero. Annual means and standard deviations were also calculated and displayed on histograms of the percent frequency of occurrence of cover types used each year so as to identify individual cover types with significant differences between the years as well as significant usage relative to other available cover types within each year.

All snowtracking data points, including both 15 minute "habitat points" and the "activity points" collected for behavioral observations including predation attempts, scent marking, rest sites, etc. were used in this analysis. This added greater weight to habitats with greater amounts of activity, thus identifying specific biotic communities with important ties to fox ecology. But data from the Muddy Creek and Ghost Creek transects that were not consistently sampled both winters were excluded from this analysis.

Here I also used Fisher's exact tests and side-by-side histograms overlaid with annual means and standard deviations to assess interannual variance in food habits. In assembling its contingency tables, counts for a given food type were only recorded if that food type comprised at least 25% of content recovered from the sample to avoid bias from indigestable food items consumed in large quantities, such as ungulate hair. This threshold was chosen after examining bimodal distributions of the proportions of food item contents (both high and low proportions) versus the right-skewed unimodal distributions of the proportions of debris (only low proportions) that generally reached maximum values around 25%. Although graminoid vegetation may be a food item during the growing season, its uptake in the winter is likely incidental as a result of eating

cached food items, small mammal nests, etc. during the winter when nutritional content is low, making it a non-food item in that case.

Comparing this food habit analysis with the preceding cover type analysis provided biological interpretations for statistical variance observed, especially with respect to cover types associated with particular food resources.

RESULTS

RSPF Models

Probabilistic response surfaces from all use datasets produced a band of high RSPF values between elevations of 2,550 m and 2,950 m (**Figure 2.5).** This elevational band is characterized by montane and subalpine forests and xeric and mesic parklands. Elevations above this band, dominated by alpine tundra and rocks, and below it, dominated by sagebrush meadows and montane forests, received lower RSPF values. There is a patch of low RSPF values within the band of otherwise high values corresponding with the Beauty Lake snowtracking transect where no fox use observations were recorded in six surveys. Even though this "no use" data was not a direct input, the model's output indicates that it captured some of the same environmental factors affecting decreased selection of thick forests like that near Beauty Lake.

Response surfaces become more detailed and have greater consistency between datasets with sample sizes greater than $n = 200$, indicating that this is the ideal minimum sample size for this analysis. Measures of model fit (**Table 2.2** and **Table 2.3**) likewise improved with sample size. The effect of low sample size seemed to be greater than that of minor differences in collection methodology between subsets, therefore I selected the response subset using all GPS 15 minute relocations and all snowtracking 15 minute habitat points (n = 382) as the most robust sample of habitat use by red foxes here. I then removed the not significant (p = 0.497) 2nd order polynomial slope term to run a most parsimonious model fit to this most robust dataset–a not significant (p = 0.127) 1st order

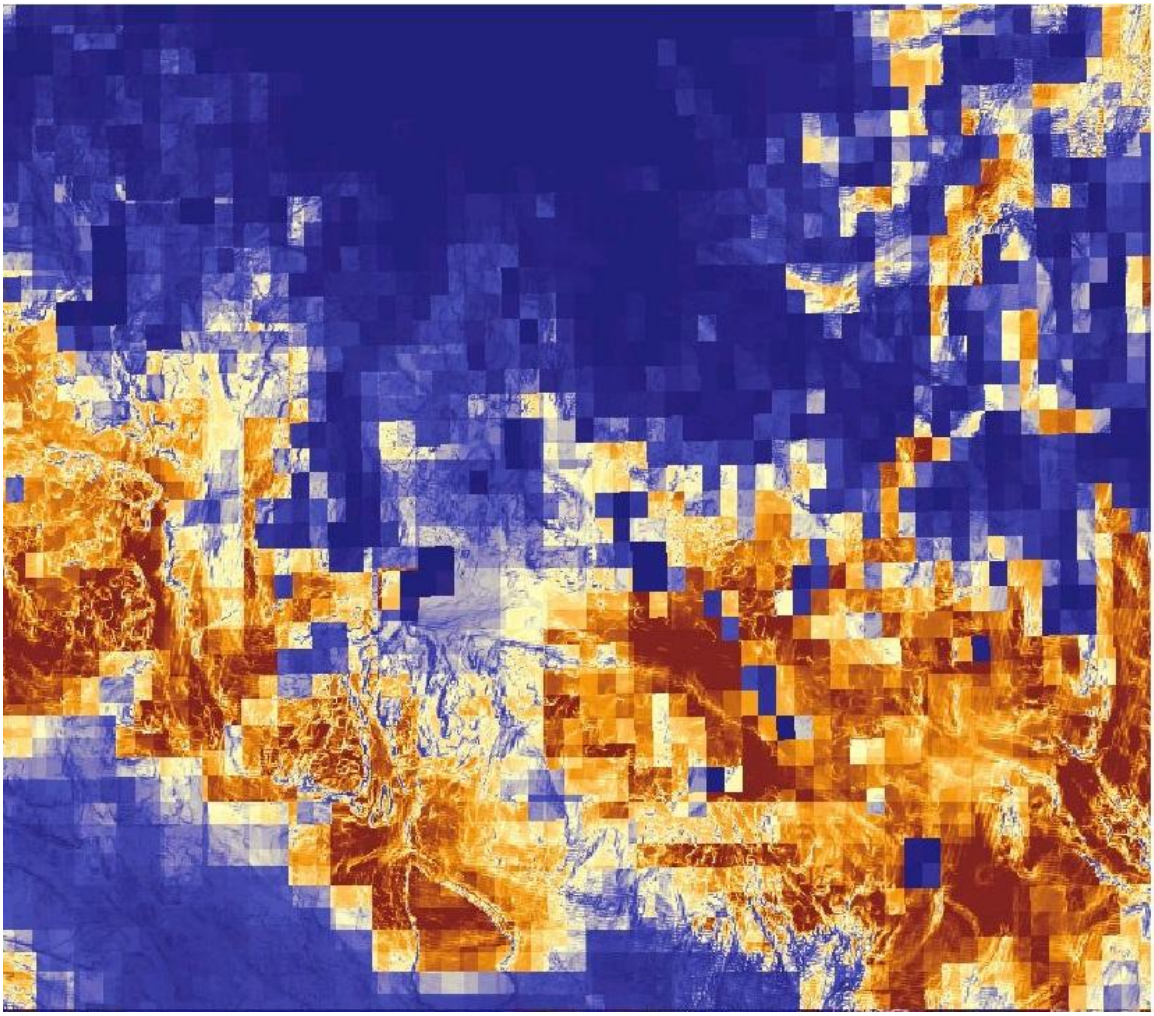

Figure 2.5: Probabilistic response surface generated in the final RSPF model using all available use inputs (n = 328) and both 1st and 2nd order terms of NPP, snow depth, and slope covariates. Blue indicates low RSPF values, and red indicate high RSPF values. The band of high RSPF values between elevations of 2,550 m and 2,950 m captures habitats where fox use was observed, while patches of low RSPF values within that band are consistent with areas that were surveyed but where fox use was not recorded.

snow depth term was retained since its subsequent 2nd order term did have a significant effect (**Table 2.2**). Yet this most parsimonious model produced poorer measures of model fit than the model using all covariate terms, including lower log-likelihood estimates and a less significant Hosmer-Lemeshow goodness of fit estimate. Therefore,

Response	n	NPP (p)	NPP^2 (p)	snow (p)	snow^2 (p)	slope (p)	slope^2 (p)
SnowTracking 2013	92	0.095	*0.039*	*0.047*	0.055	0.113	0.120
ST 2014 (No Muddy/Ghost)	54	0.347	*0.015*	0.519	0.190	0.781	0.126
ST 13 /14	238	*0.038*	*0.025*	0.154	*0.005*	0.050	0.300
ST 13/14 (NM/G)	146	*< 0.001*	*0.000*	0.473	*< 0.001*	*< 0.001*	*0.001*
GPS 2013 (No May)	110	0.222	*0.005*	*0.004*	*< 0.001*	0.292	0.145
GPS 2013 (NM) + ST 2013	202	*< 0.001*	0.976	*0.044*	*< 0.001*	0.928	0.354
GPS 2013 (NM) + ST 13/14 (NM/G)	256	*0.002*	*< 0.001*	0.290	*< 0.001*	0.115	*0.015*
GPS 2013 (NM) + ST 13/14	348	*0.012*	*0.011*	0.272	*0.020*	0.195	0.682
GPS 2013 + ST 13/14	**382**	***< 0.001***	***0.001***	**0.127**	***< 0.001***	***0.005***	**0.497**
Parsimonious GPS13 + ST13/14	382	*< 0.001*	*< 0.001*	*0.022*	*< 0.001*	*0.008*	NA

Table 2.2: Significance of univariate polynomial terms in RSPF component GLMs. Significant results ($p < 0.05$) are italicized, and the final model is in bold.

the final RSPF model chosen to best represent actual red fox resource selection used all of the available response inputs and all of the available covariate terms (**Figure 2.5**).

Bootstrapped values of GLM simulations for each covariate identified quantitative univariate values with positive or negative effects on RSPF values. For NPP ($p = 0$, AUC = 0.509), scaled RSPF peaked between 12 g C m^{-2}/year and 17 g C m^{-2}/year; for snow depth ($p = 0.003$, AUC = 0.507), scaled RSPF peaked between 600 cm and 1,500 cm; and for slope ($p = 0.01$, AUC = 0.552), scaled RSPF started high then fell off at slopes steeper than 15°.

Response	n	AIC	Log-Likelihood GLM	Log-Likelihood Nelder-Mead	Hosmer-Lemeshow (p)
SnowTracking 2013	92	-40.404	22.441	27.202	< 0.001
ST 2014 (No Muddy/Ghost)	54	-13.835	13.572	13.918	0.573
ST 13 /14	238	-68.903	36.640	41.452	0.000
ST 13/14 (NM/G)	146	-84.890	48.464	49.445	*0.068*
GPS 2013 (No May)	110	-22.803	12.473	18.402	0.005
GPS 2013 (NM) + ST 2013	202	-60.440	17.568	37.220	*0.012*
GPS 2013 (NM) + ST 13/14 (NM/G)	256	-48.465	30.387	31.233	*0.130*
GPS 2013 (NM) + ST 13/14	348	-84.958	48.602	49.479	0.002
GPS 2013 + ST 13/14	**382**	**-132.138**	**71.251**	**73.069**	***0.178***
Parsimonious GPS13+ST13/14	382	-123.158	65.861	67.579	0.041

Table 2.3: Fit summaries from nine RSPF models testing subsets and combinations of response inputs, and a tenth most parsimonious model. Significant results ($p > 0.05$) are italicized, and the final model is in bold.

The RSPF equation for the final model is:

$$\text{RSPF} = -4.8218 + \frac{-0.1323(SNOW\ DEPTH - 1113.1438)}{447.4867} + \frac{-0.4213(SNOW\ DEPTH - 1113.1438)^2}{447.4867} + \frac{1.2529(NPP - 12.5834)}{4.2760} + \frac{-1.0068(NPP - 12.5834)^2}{4.2760} - \frac{0.2263(SLOPE - 15.9000)}{12.4731} + \frac{-0.0479(SLOPE - 15.9000)^2}{12.4731}$$

Euclidean Distance Method of Assessing Non-Random Selection

In the $K = 3$ analysis (**Figure 2.6**), strong selection for forested habitats (-0.354) was detected in the most robust dataset used in the final RSPF run: the mean observed distance from forest habitats was 36.18 m compared to 49.16 m expected from available forest habitat (**Table 2.4**). Yet selection for non-forested habitats (-0.274) and edge habitats (-0.350) was also strong, and the differences between these ratios was not significant. In the $K = 6$ analysis (**Figure 2.7**), the LPDF classification received the strongest selection (-0.312) followed by Mesic (-0.295), SF (-0.270), Sage (-0.198), Rocks (-0.086), and Xeric (-0.057) (**Table 2.5**).

Different and conflicting results were produced with the snowtracking and GPS telemetry datasets individually. Snowtracking indicated greater selection for non-forested habitats over forested habitats both winters with a significant ($\chi^2 = 17.562$, $p < 0.001$) difference in 2014. But 2013 GPS data indicated significant ($\chi^2 = 30.698$, $p < 0.001$) selection of forested habitats (-0.628) next to a positive mean distance ratio (0.986) for non-forested habitats resulting from a mean distance (111.67 m) much greater than expected (56.24 m). Selection for edge habitats was also strong in the snowtracking data for both years (-0.529) while it was weak (0.288) in the GPS data.

In the $K = 6$ analyses comparing these sampling methods, snowtracking data from 2013 and 2014 combined showed selection for the Xeric classification to be the strongest (-0.697), followed by Mesic (-0.482), SF (-0.327), Rocks (-0.216), and Sage (-0.152). LPDF received the only positive mean distance ratio (0.083) indicating weak selection in this snowtracking dataset, yet it received the most negative mean distance ratio (-0.686)

indicating the strongest selection in the GPS dataset, followed by Sage (-0.561) and SF (0.376). The non-forested Mesic (0.282), Rocks (0.448), and Xeric (0.970) classifications in turn showed increasingly weaker selection in the GPS dataset. These substantial differences may be due to sampling effects and behavioral biases from the response data collection methods as well as the error rate in the GAP data, all compounded by the high frequency of response inputs near habitat/classification edges.

Error Rates and Validation of GAP Land Cover Data

The 30 m GAP land cover data used to extrapolate Euclidean distance metrics from both used and available inputs in the $K = 3$ selection analysis had a high mean Type I error rate (62%) for non-forested classifications (Xeric, Mesic, and Sage) within 30 m of the forest edge (**Figure 2.8**). Type II errors were moderately high (33%) across all classifications except Sage within 30 m of the forest edge. Beyond 30 m, both error rates declined substantially for non-forested habitats resulting in significant variation ($\chi^2 = 23.338$, $p < 0.001$) between these distance-from-edge subsets. Error rates within class (forested or non-forested), however, were roughly 50% across all land cover classes and distance-from-edge subsets.

Land cover classification error for the 30 m GAP dataset, as validated with on-the-ground observations of cover type (Despain 1990) collected while snowtracking, is therefore greatest in distinguishing forested and non-forested habitats within 30 m of the edge. This makes sense since that is the resolution of the remote sensing data product, but it is important since 73% of fox habitat use records are within 30 m of the

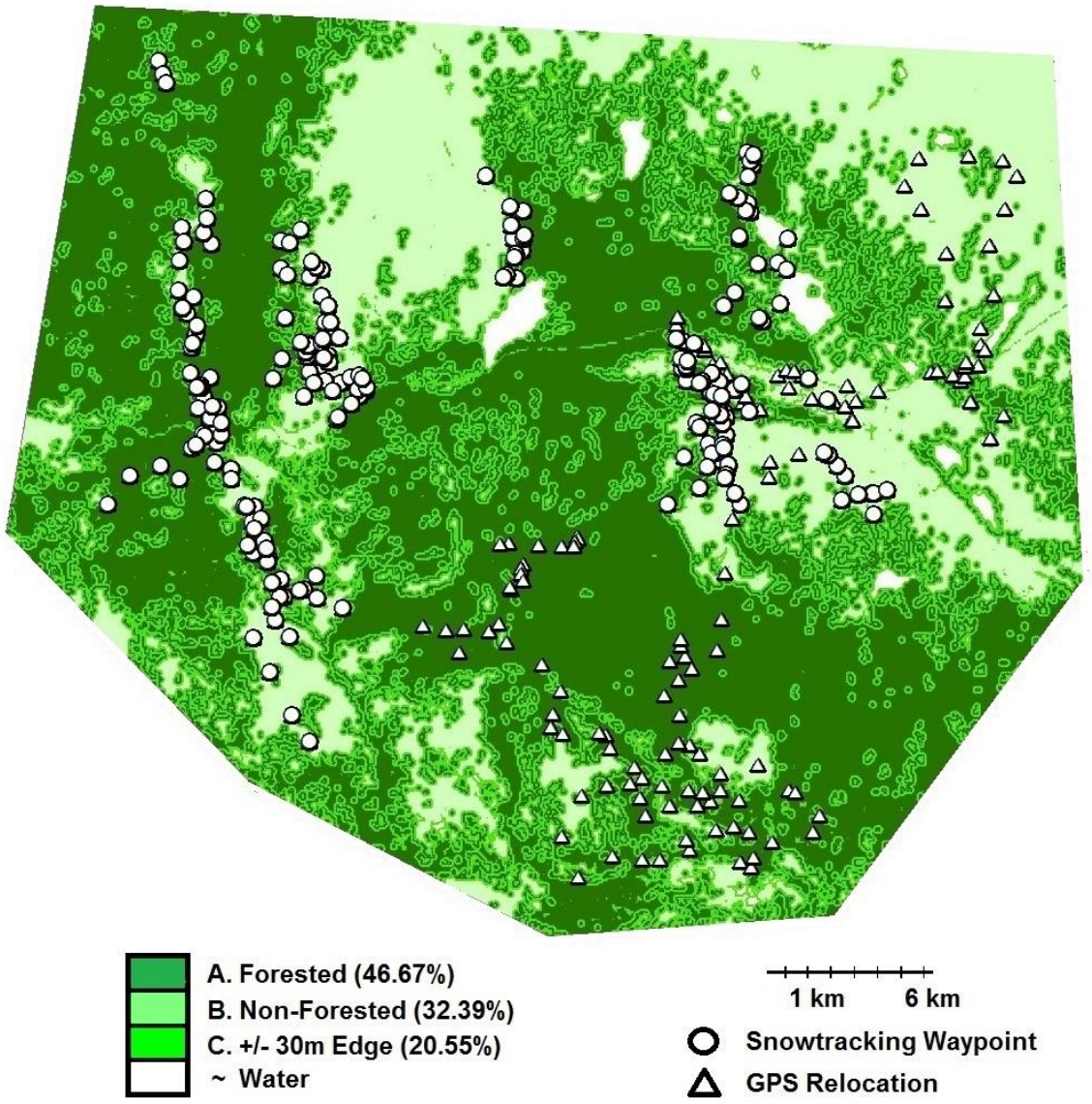

Figure 2.6: *K* = 3 land cover classifications from 30 m GAP data and the response data used to assess habitat selection.

edge. That means that 40% of Xeric/Non-Forested classifications used in the habitat selection analyses may have been affected.

Response	n	A. Forest	χ^2 / p	B. Non-Forest	χ^2 / p	C. Edge
Snowtracking 2013	92	36.18 -0.264	2.355 0.125	24.66 -0.562	*0.202* *0.653*	45.37 -0.485
Snowtracking 2014	54	39.67 -0.193	17.562 < 0.001	8.33 -0.852	7.213 0.007	37.61 -0.573
GPS 2013 (15 Minute)	110	18.27 -0.628	30.698 0	*111.67* *0.986*	17.323 < 0.001	*113.36* *0.288*
GPS 2013 (12 Hour)	108	23.75 -0.567	10.966 < 0.001	*74.59* *0.141*	5.842 0.016	77.30 -0.117
GPS + Snowtracking	382	31.75 -0.354	*1.434* *0.231*	40.81 -0.274	*0.001* *0.977*	57.27 -0.35
Expected	**181k**	**49.16**	**NA**	**56.24**	**NA**	**87.59**

Table 2.4: Mean distance (top) and ratio of observed/expected distance (bottom) for each of three land cover classifications analyzed using the Euclidean distance method to assess non-random selection (Conner and Plowman 2001). Differences between forest and non-forest ratios and between the most selected category and edge ratios were assessed with chi-squared tests.

Interannual Variance in Habitat Use and Food Habits

Habitat (Cover Type) Use

Eleven cover types were observed in the snowtracking data excluding the low elevation transects (Muddy Creek and Ghost Creek) that were only sampled in 2014 (**Table 2.6**). Four of these (LP2, WB0, mesic, and water) were only observed in 2014. This resulted in a lower median percent frequency of occurrence and a greater standard deviation in 2013 than in 2014 (**Figure 2.9**).

A Fisher's exact test indicated significant ($p = 0.0162$) variance in cover type usage between the two winters. This may be attributed to the differences in used and unused cover types as well as a significant spike in the use of spruce-fir (SF) cover types

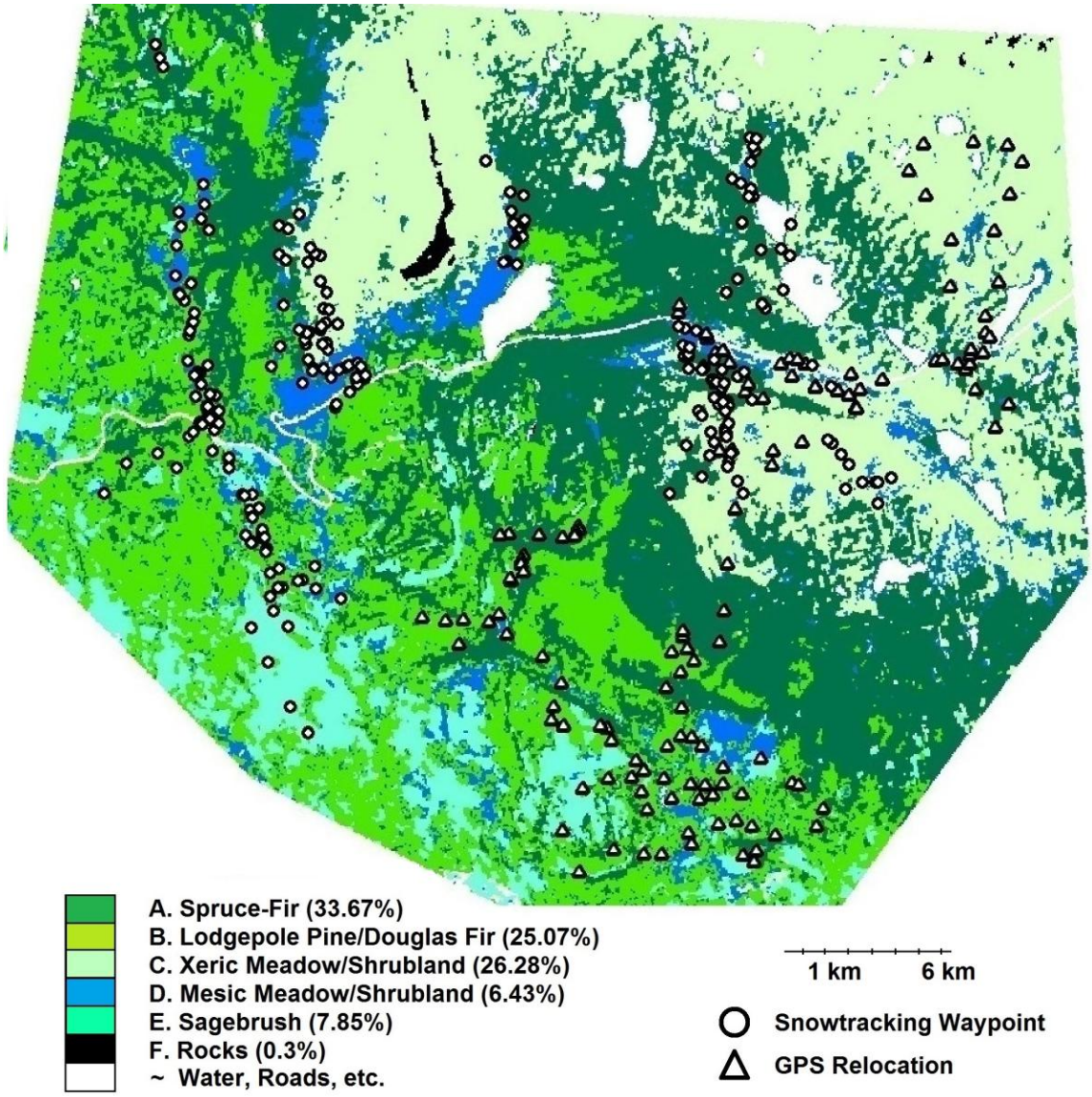

Figure 2.7: $K = 6$ land cover classifications from 30 m GAP data.

in 2013 that leveled off in 2014. The percent frequency of occurrence of SF in 2013 was 29.4872%, which is greater than two standard deviations from the 2013 median, while it was 11.5385% in 2014, less than two standard deviations from the 2014 median. There was also slightly greater use of climax whitebark pine (WB) in 2013 compared to 2014,

Response	n	A. SF	B. LPDF	C. Xeric	D. Mesic	E. Sage	F. Rocks
ST 2013	92	57.05 -0.315	*589.3* *0.051*	67.27 -0.649	80.06 -0.532	849.6 -0.163	2258.02 -0.207
ST 2014	54	54.42 -0.347	*607.62* *0.083*	42.01 -0.781	103.48 -0.395	881.2 -0.132	2181.93 -0.233
ST All	146	56.08 -0.327	*596.08* *0.063*	57.93 -0.697	88.72 -0.482	861.29 -0.152	2229.88 -0.216
GPS 2013 (15 Minute)	110	51.99 -0.376	176.34 -0.686	*377.03* *0.970*	*219.41* *0.282*	445.4 -0.561	*4122* *0.448*
GPS 2013 (12 Hour)	108	47.59 -0.429	429.59 -0.234	*240.39* *0.258*	163.69 -0.044	713.67 -0.297	*3631.95* *0.276*
GPS (15) + ST 2013	202	54.29 -0.348	364.43 -0.350	*235.95* *0.233*	155.94 -0.089	629.49 -0.380	*3273.06* *0.150*
GPS (15) + ST All	382	60.78 -0.270	386.09 -0.312	180.58 -0.057	120.65 -0.295	814.39 -0.198	2600.73 -0.086
Expected	**181k**	**83.3**	**560.86**	**191.42**	**171.14**	**1015.3**	**2846.04**

Table 2.5: Mean distance from (top) and ratio of observed/expected distances (bottom) for each of six land cover classifications assessing non-random selection where negative values indicate greater use than expected from availability.

while 2014 saw greater use of the mid-successional cover types LP2, LP3, and WB2 compared to 2013.

There were also interannual differences in the use of edge habitats between forested and non-forested cover types (**Figure 2.2**). In 2013 there was an increase in use of forested cover types 120 m or farther from the edge, while 2014 saw substantially greater use of non-forested cover types 120 m or farther from the edge as well as distances that were closer to the edge.

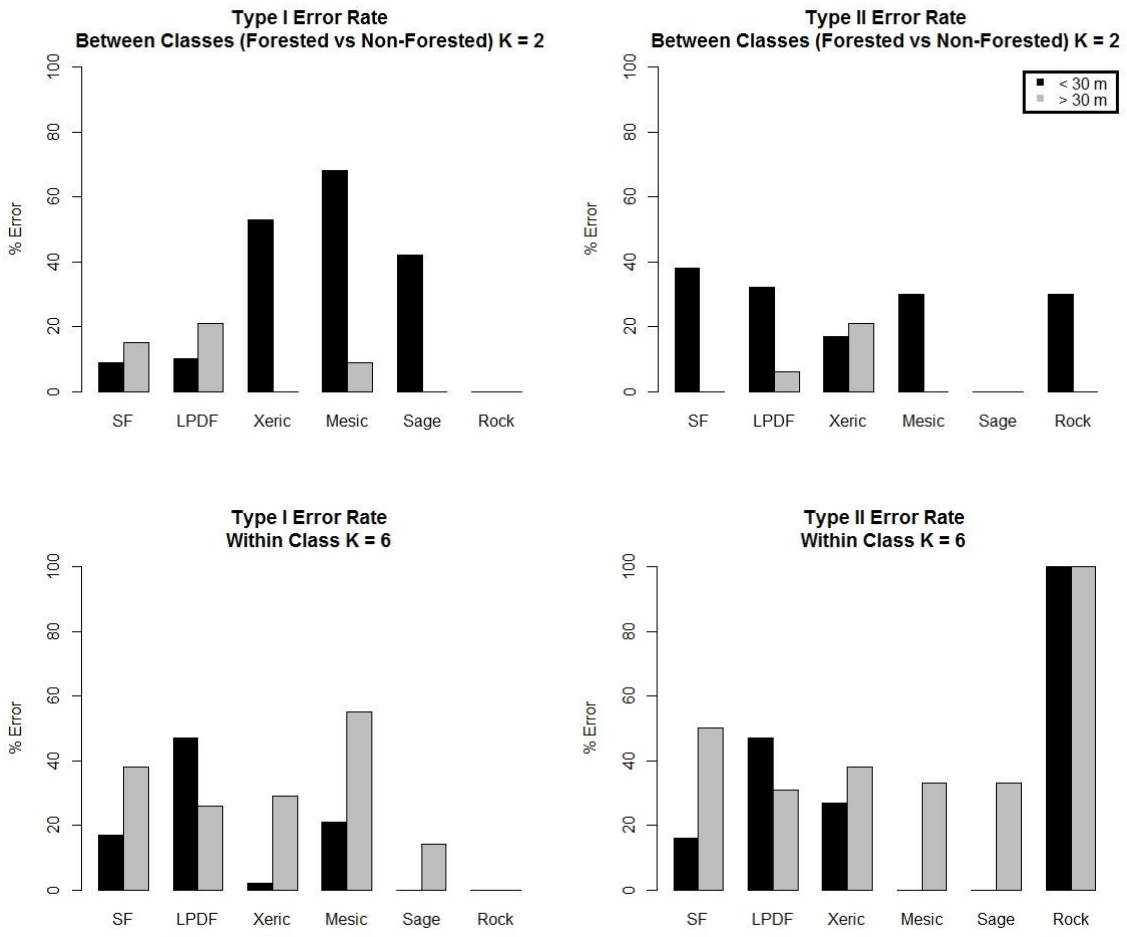

Figure 2.8: Percent error calculations for 30 m GAP land cover classifications validated with cover type (Despain 1990) observations recorded while snowtracking.

Food Habits

Excluding non-food items and unknown items, 10 species were identified in scats collected in winter 2013 (**Table 2.7**). This does not include small rodents such as voles (Arvicolinae), deer mice (*Peromyscus maniculatus*), or shrews (*Sorex* sps.) since they could not be identified by hair alone, although this was a substantial portion of total fecal content. No intact jaws or skulls were recovered from scats collected in the winter, yet

Despain (1990) Cover Type	Cover Type Code	Ct. 2013	% Freq. 2013	Ct. 2014	% Freq. 2014
Climax Spruce-Fir	SF	23	29.49	6	11.54
Climax Whitebark Pine	WB	14	17.95	4	7.69
Mid-Successional Whitebark Pine	WB2	11	14.10	11	21.15
Early-Successional Whitebark Pine	WB0	0	0	2	3.85
Mid-Successional Lodgepole Pine	LP2	0	0	2	3.85
Late-Successional Lodgepole Pine	LP3	1	1.28	2	3.85
Rock	Rock	3	3.85	1	1.92
Road	Road	2	2.56	3	5.77
Open Water	Water	0	0	1	1.92
Mesic Meadows	Mesic	0	0	1	1.92
Xeric Meadows	Xeric	24	30.77	19	36.54

Table 2.6: Despain (1990) cover types recorded while snowtracking red foxes in 2013 (red) and 2014 (blue) including count and percent frequency occurrence for each year.

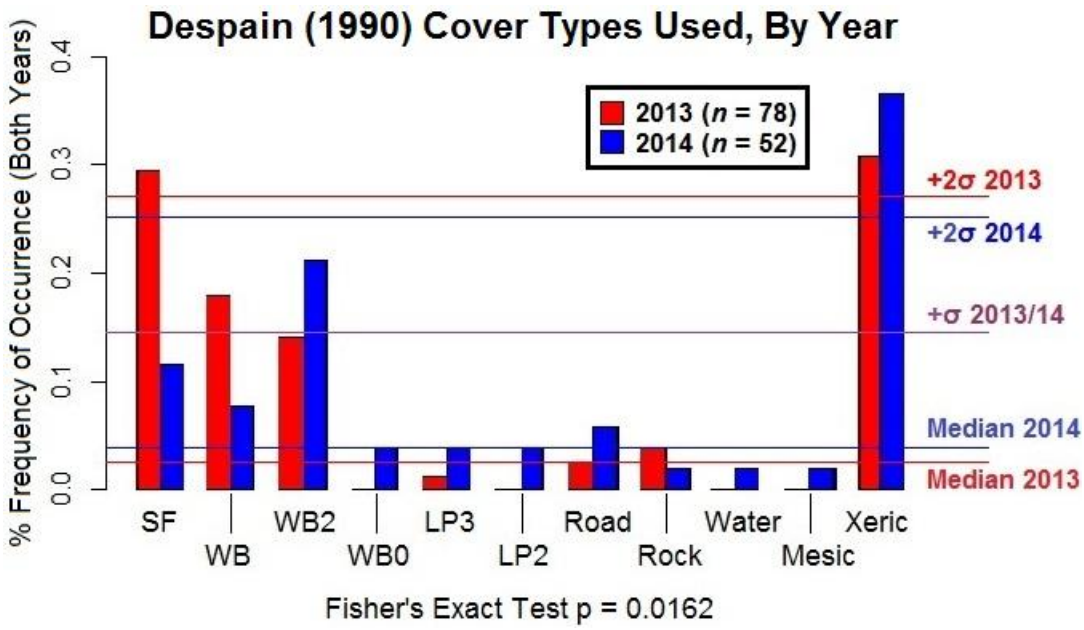

Figure 2.9: Percent frequency of occurrence of eleven cover types (Despain 1990) observed while snowtracking red foxes in 2013 (red) and 2014 (blue). A Fisher's exact test (p = 0.0162) indicates significant variance between the years, driven by significantly greater use of the spruce-fir (SF) cover type in 2013 than in 2014.

they were frequently recovered from scats collected the following summer. This seasonal difference may be due to greater digestive efficiency in the winter driven by lower food availability and greater caloric demands (Kelley and Garton 1997). In summer 2013, seven individual meadow voles (*Phenacomys intermedius*), one montane vole *(Microtus montanus*), and one vagrant shrew (*Sorex vagrans*) were identified in 13 samples.

This count also does not include birds: small, unidentifiable feathers were found in 2013 alone, predominantly in trace amounts (minimum = 1%, mean = 2.48837%, maximum = 25%) suggesting that many of these feathers may have been incidental uptake when consuming cached items along with other forest debris. Yet two records with 20% and 25% bird, respectively, also had bone fragments, while a dusky (blue) grouse (*Dendragapus obscurus*) predation was recorded in 2013 snowtracking, so these (and likely other birds) were also food items.

In 2014, only six food items were identified to species. Unidentifiable small mammals (ie. voles) and unknown food items as well as vegetation and debris were also recorded. No bird was recorded in 2014. In categorizing these data, I considered whitebark pine (PIAL), snowshoe hare (LEAM), graminoid vegitation (Veg), and birds individually, and combined counts for northern pocket gophers (*Thomymus talpoides*) and microtine rodents (Micro), deer and elk (Cervid), other mammals (Other).
A Fisher's exact test indicated significant ($p < 0.001$) variance in food items used between the two winters (**Figure 2.10**). This may be attributed to a significant spike in whitebark pine use in 2013 when pine nuts were found in 14 out of 30 scats (46.6667%) comprising a minimum of 20%, a mean of 61%, and a maximum of 97% of those scats'

Species Name	Common Name	Ct. 2013	% Freq. 2013	Ave. % Cont.	Ct. 2014	% Freq. 2014	Ave. % Cont.
Pinus albicaulis	Whitebark Pine	19	44.18	50.42	1	2.04	4
Lepus americanus	Snowshoe Hare	10	23.26	55.2	22	44.9	55.2
Thomomys talpoides	Northern Pocket Gopher	5	11.63	32.4	12	24.49	66.58
Microtine	All Voles	23	53.49	51.78	6	12.24	31.67
**Phenacomys intermedius*	Meadow Vole	7	16.28	83.67	0	0	0
**Microtus montanus*	Montane Vole	0	2.33	60	0	0	0
Spermophilus sps.	Ground Squirrel	3	6.98	36.67	4	8.16	36.67
Cervus elaphus	Elk	10	23.26	26.1	9	18.37	93.78
Odocoileus hemionus	Mule Deer	1	2.33	59	1	2.04	15
Zapus princeps	Western Jumping Mouse	1	2.33	2	0	0	0
Neotoma cinerea	Bushy-tailed Woodrat	2	4.65	24.5	0	0	0
Tamiasciurus hudsonicus	Red Squirrel	3	6.98	3.67	0	0	0
Tamias sps.	Chipmunk	1	2.33	2	0	0	0
Marmota flaviventris	Yellow-bellied Marmot	2	4.65	52.5	0	0	0
Ochotona princeps	Pika	2	4.65	4	0	0	0
Sorex vagrans	Vagrant Shrew	1	2.33	15	0	0	0
Vaccinium scoparium	Grouse Whortleberry	1	2.33	3	0	0	0

Table 2.7: Food items recorded in winter and summer 2013 (red) and winter 2014 (blue) scats, including the count, percent frequency of occurrence, and average proportion of content for each species for *all* observations. Voles identified to species (*) were only collected in summer 2013, and no summer records were included in the winter food habit analysis, nor were individual observations with proportions less than 25%.

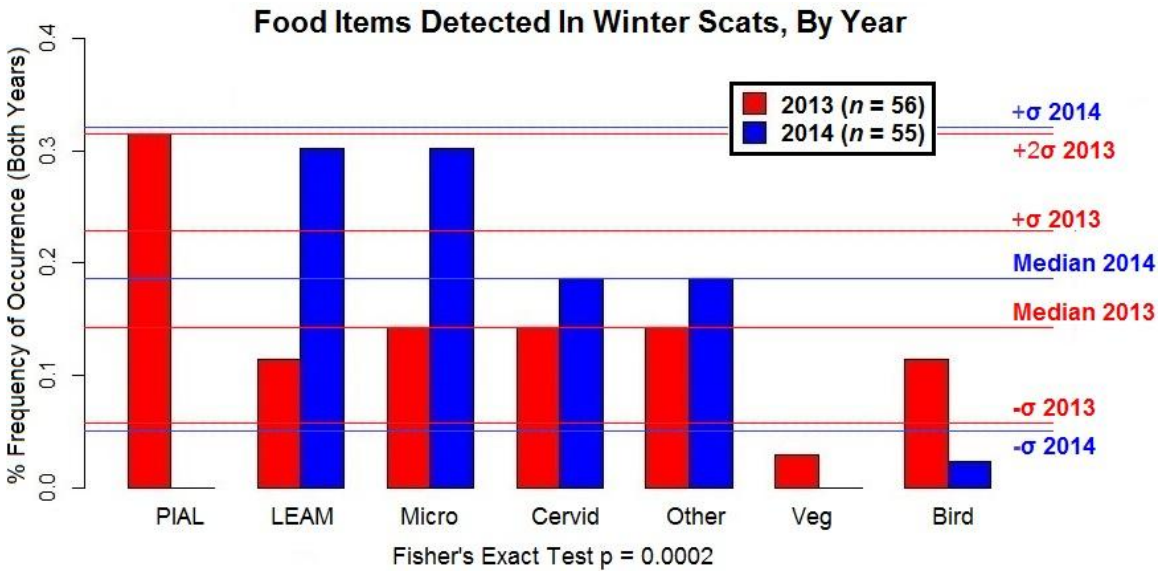

Figure 2.10: Percent frequency of occurrence of seven food item categories detected in at least 25% of individual red fox scat contents in winter 2013 (red) and winter 2014 (blue). A Fisher's exact test ($p < 0.001$) indicates significant variance between the two years, driven by significantly greater use of whitebark pine in 2013 than 2014.

contents. Its percent frequency of occurrence in 2013 was 31.4286%, which equals two standard deviations (8.5714%) greater than the 2013 median (14.2857%) for all food groups. Pine nuts were also found in summer 2013 in five out of 13 scats collected (38.4615%) comprising a minimum of 5%, a mean of 20.8%, and a maximum of 40% of those scats' contents, although these observations were not included in the winter food habit analysis.

In 2014, only a trace amount (4%) of pine nut was found in one sample, and since it comprised less than 25% of that sample's content, it was not considered in further analysis. Therefore, whitebark pine consumption was not recognized in 2014. This corresponded with a substantial decrease in vegetation and debris, which were both found

in 56.6667% of samples collected in 2013 with mean individual proportions of 22.2353% and 11.2353%, respectively, while vegetation was found in 35.7143% of samples and debris in only 9.5238% of samples in 2014 with mean individual proportions of 6.6% and 27%, respectively. 2014 also saw substantial increases in the snowshoe hare and microtines detected as the percent frequency of occurrence for both doubled compared to 2013. Cervid and other categories only saw slight increases in 2014 compared to 2013.

DISCUSSION

Multiple analyses show that red foxes at high elevations on the Beartooth Plateau continue to select forest habitats in an environment with little or no competition from resident coyotes. The probabilistic response surface of a well fit RSPF model built with sufficient numbers of independent samples and significant covariate terms captures large portions of subalpine forests in the study area. Mean Euclidean distance ratios calculated using the same robust response data indicate greater selection for forested habitats than for non-forested habitats. And additional evidence comes from the fecal content analysis: Intact skulls needed to identify voles to species were only recovered in the summer due to seasonal differences in digestive efficiency, but when they were, seven out of eight (87.5%) belonged to meadow voles. Meadow voles prefer forested habitats and are found here at the southern extent of their range spanning the boreal forests of Canada (Foresman 2012). Only one montane vole, a species that prefers wet meadows and is reported to be common on the Beartooth Plateau, was recovered, while no identifiable remains from other vole species known to reside there and presumably be available—the boreal red-backed vole, the long-tailed vole (*Microtus longicaudus*), or the water vole (*Arvicola richardsoni*)—were recovered (Pattie and Verbeek 1967). Selection of a prey item associated with boreal forests thus supports a like association for its predator.

Coyotes are common at and below elevations of 2,000 m in the GYE, and competition with them and even predation by them no doubt affects sympatric red fox populations. Forest habitats may help such foxes avoid competition. But the continuation of forest habitat selection by foxes in an environment where coyotes are

excluded by deep snow indicates that drivers beyond avoidance mechanisms affect forest habitat selection in both populations.

Yet I also detected consistently strong selection of edge habitats, so its implications for accurate as well as relevant habitat selection inference must be considered. Classification errors in the land cover data are clearly compounded by edge use, especially since the 30 m resolution of the remote sensing products essentially overlaps the forest to non-forest ecotone as the foxes were observed to use it. While this likely had an even greater effect on the 500 m NPP and 1 km SnoDAS covariates used in the RSPF models, it is interesting to observe that their output assigned the highest RSPF values to edges and areas with the most heterogeneous land cover, thus producing similar results using different variables and methods.

Considered individually, different data collection methods produced different results. Snowtracking data from both years showed strong selection for non-forested habitats, where Type I errors were most common, as well as edge habitats, where Type I errors occurred nearly twice as frequently as in non-edge habitats. The GPS dataset, in contrast, showed weak selection for both non-forested and edge habitats. This difference could an artifact of a proximity bias in the snowtracking data from transects located on or near forest edges. It may also be the result of behavioral differences in animals being observed through snowtracking versus those observed with GPS telemetry. Foxes using edge habitats and the roads and trails that I used for transects are frequently engaged in scentmarking behavior since territorial boundaries often coincide with natural and anthropogenic transitions, whereas a GPS collar continuously collecting data over a long

time period would independently sample a wider range of behaviors. But even though this GPS dataset produced significant results, it should not be considered on its own since it was collected from just one animal owing to the failure of all the other GPS collars. Therefore it is not fully independent as the animal's social status, age, and condition as well as seasonal and interannual behavioral differences would have affected use, which is especially true given that winter's whitebark pine cone availability. So as with the RSPF model, the dataset spanning multiple years and multiple collection methods is probably the most robust response data available for this Euclidean distance approach.

But with this significant use of edge habitats, neither the classification errors nor the difference in selection of forested over non-forested cover types may matter since foxes are selecting a mix of both habitats within their ecotones anyway. I therefore suggest that overall resource scarcity in the alpine environment–demonstrated by the 87 km^2 area that 5.9 kg M800 covered in just three months to search for resources–and greater resource potential for adaptable foxes in more heterogeneous edge habitats are among the most important factors driving habitat selection at high elevations. Resource scarcity and the adaptability of individual foxes will ultimately drive foxes to seek resources wherever they are available: in other parts of the world, foxes have been recorded traveling far outside their usual home ranges to take advantage of novel food sources such as spawning salmon in Japan or roe deer fawns in Norway (Panzacchi *et al*. 2009, Tsukada 1997). This should be considered alongside intrinsic preferences and competitive pressures affecting observations of forest habitat selection in this and other montane populations.

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There was substantial interannual variance in the snow conditions and food availability during the two winters of data collection, and that affected habitat and resource selection by the foxes being studied. The snowpack was well below average in 2013 then well above average in 2014, affecting access to food resources both above the snow, like snowshoe hare, and below it, including caches and small mammals in the subnivean space. But perhaps the most significant difference between 2013 and 2014 was in the availability of whitebark pine nuts.

Whitebark pine, along with other plants producing nutrient rich seeds highly prized by seed predators, exhibit interannual variance in seed production known as mast seeding (Crone *et al*. 2011). Mast seeding discourages seed predators from residing near the food source where they may consume most if not all of the seeds, thus improving the probability of regeneration (Keane *et al*. 2012). As this is an important food resource for grizzly bears (*Ursus arctos*), the Interagency Grizzly Bear Study Team (IGBST) monitors annual whitebark pine cone production at plots across the GYE including the Beartooth Plateau. The summer of 2012 was reported to have "generally good cone production" (Haroldson and Podruzny 2012), and the following winter significant frequencies of whitebark pine nuts were recovered from red fox scats. Snowtracking that winter also led us to three excavated red squirrel middens, evidence that red foxes, like grizzly bears, obtain this nutritious food source through kleptoparasitism of red squirrel middens. Individual middens can contain as many as 1,000 cones, each with as many as 50 food calories (Keane *et al.* 2012, Reinhart and Mattson 1989).

The summer of 2013, however, had "generally poor cone production" (Haroldson 2013), and the percent frequency of occurrence of pine nuts in fox scats fell to zero the following winter. In response, foxes consumed more snowshoe hare and subnivean mammals like voles and pocket gophers during winter 2014. The increased use of microtine rodents may be attributed to greater availability that winter–I recorded an anecdotal observation that there were more above ground vole dens, which are often used as a proxy for vole census population size (Robert Crabtree, personal communication), in summer 2013 than in summer 2012. Yet I did not observe obvious differences in the distribution or abundance of snowshoe hare tracks between the two winters while snowtracking, suggesting that environmental variance including lower whitebark pine availability, deeper snow, and/or other factors may have influenced foxes to switch to greater snowshoe hare consumption.

The dramatic variance in whitebark pine use is not surprising in itself since the availability of whitebark pine was so different between the two winters, nor is the use of this highly nutritious food resource by this highly adaptable species particularly surprising. What is surprising though is that it coincides with a significant spike in spruce-fir cover type usage in 2013 that likewise falls off in 2014. The role of red squirrels in the fox:whitebark relationship helps explain this correlation between habitat use and food habits.

Because of mast seeding, pure whitebark pine stands are generally poor red squirrel habitat as they lack sufficient food resources to sustain squirrels during low cone production years (Reinhart and Mattson 1989). Subalpine spruce-fir stands, on the other

hand, have a greater diversity of more consistent albeit less nutritious food sources along with a significant whitebark pine component (Despain 1990, Reinhart and Mattson 1989). This makes spruce-fir cover types the best red squirrel habitat and the most likely place that red foxes will find squirrel middens with whitebark pine cones. Such a correlation of habitat use and resource availability implies that the significant selection of spruce-fir cover types in 2013 was driven by the availability of whitebark pine nuts that winter. This in turn suggests that foxes were not only using this novel food source, but that they changed their habitat use behavior in response to its availability.

This adds red fox to the list of over eight mammals known to forage on whitebark pine seed (Lorenz *et al*. 2008), yet this is not a complete list. I observed two American marten scats that contained whitebark pine nuts on the Beauty Lake snowtracking transect on March 28, 2013, and marten scats containing pine seeds have also been reported in previous studies (Hargis and McCullough 1984, Zielinski and Duncan 2004). Humans have likewise consumed whitebark pine nuts in the region since prehistoric time: archaeologists recently found 13 villages dated over 2,000 years old at elevations above 3,200 m in the GYE's Wind River Mountains (Stirn 2014). All were located near whitebark pine stands, and all contained grind stones and other artifacts associated with nut and seed processing. Indeed, the Tipatikka band of the Shoshone tribe, kin to Yellowstone's Tukudeka or "Sheep Eaters", are also known as the "Pinenut Eaters".

The interannual difference in whitebark pine nut availability and its significant effect on red fox habitat selection may have influenced the decline in forest cover type frequencies observed in snowtracking from 64% in 2013 to 51% in 2014 (**Figure 2.11**).

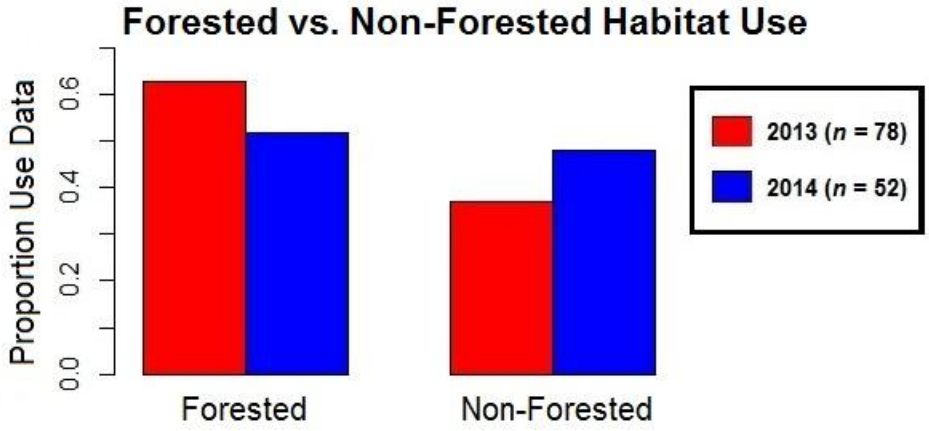

Figure 2.11: Forested and non-forested habitat use between 2013 (red) and 2014 (blue).

Yet it did not seem to have a major impact on the overall selection of forested over non-forested habitat, especially after comparing snowtracking subsets from 2013 when whitebark was available and 2014 when it was not. Interannual environmental variance, therefore, may have influenced *how* red foxes on the Beartooth Plateau used forest habitats, but it did not change *that* forest habitats were selected.

While the adaptability of individual foxes fails to support the hypothesis that selection of a specific habitat type, namely subalpine forests, effects a reproductive barrier between native and non-native foxes, this novel foraging strategy could theoretically have evolutionary consequences. Whitebark pine are restricted to high elevations where non-native foxes are excluded, therefore whitebark pine foraging behavior is restricted to native foxes. Behavioral differences like this have prompted speciation in other taxa. For example, the switch from terrestrial to arboreal foraging behavior by pigeons and doves (Columbidae) resulted in morphological changes that increased specialization in the novel foraging behavior while preventing reversion to the

former behavior: an evolutionary feedback loop (Lapiedra *et al.* 2014). So whitebark pine consumption by red fox may be further evidence of the species' remarkable adaptability, or it may be a driver of differentiation at high elevation.

Forest habitats, and the resources therein, play a critical role in the life history of montane red foxes in the GYE and elsewhere. The relationship with forest habitats observed here connects the GYE population to its montane relatives and possibly its boreal ancestors, while the relationship with whitebark pine helps explain how this population persists in such an extreme environment. Yet whitebark pines are in decline across the GYE as they are threatened by mountain pine beetle (*Dendroctonus ponderosae*) and white pine blister rust (*Cronartium ribicola*) infestations compounded by drought stress (Logan *et al.* 2010), so the future of whitebark pine and its many ecological relationships is uncertain. Considering its importance to this montane fox population, the future of native foxes on the Beartooth Plateau may be uncertain as well.

EPILOGUE

SHOULD THE RED FOX POPULATION OF THE GREATER YELLOWSTONE ECOSYSTEM BE CONSIDERED A DISTINCT POPULATION SEGMENT UNDER THE UNITED STATES ENDANGERED SPECIES ACT?

There are three criteria that must be considered to determine if a population qualifies as a distinct population segment (DPS) under the U.S. Endangered Species Act (ESA): is the population discrete? is it significant? and what is its conservation status? (USFWS and NMFS 1996). To be a discrete, a potential DPS must be separate from other populations of the same taxon due to physical, physiological, ecological, or behavioral factors and/or deliminated by international governmental boundaries effecting significant differences in how it is managed. To be significant, a potential DPS must exist in an unusual or unique ecological setting, fill a significant gap in the range of the taxon, be the sole surviving natural population of a taxon that may exist elsewhere as an introduced population outside of its historic range, and/or have markedly different genetic characteristics compared to similar taxon. The conservation status of a potential DPS follows the ESA's guidelines for "threatened" or "endangered" taxa.

The exclusion of non-native mtDNA haplotypes from the high elevation red fox population in the GYE, along with exact *G* test results showing significant population differentiation between high and low elevation groups, is evidence that the high elevation population is discrete from the low elevation population whether due to physical (elevational barriers), physiological (asynchronous reproductive cycles), ecological

(adaptations to the extreme environment), or behavioral (use of forest habitats and resources such as whitebark pine seed that only occur at high elevations) factors.

Yet the high degree of gene flow between these populations shown through low F_{ST} values may counter this evidence. There are, however, precedents of DPSs in the ESA with high female philopatry as well as high male-mediated gene flow like the loggerhead sea turtle (*Caretta caretta*) (USFWS 2011). Female turtles return to natal beaches for parturition, whereas male turtles have less fidelity for natal beaches and may also mate opportunistically at sea; as a result, the mtDNA of female turtles at beaches on Boavista and San Vicente, respectively, in the Cape Verde archipelago had φ_{ST} values of 0.261 compared to microsatellite F_{ST} values of 0.025 (Stiebens *et al.* 2013). These measures of differentiation between maternally inherited mtDNA and biparentally inherited nuclear DNA mirror those that I calculated among red foxes of the GYE. And while Stiebens and colleagues (2013) had originally hypothesized that female philopatry and small population sizes would threaten loggerhead sea turtles through a reduction in genetic diversity, they instead found that such behavior helps maintain local adaptations while the synergistic interaction of asymmetric gene flow helps maintain genetic diversity. Female philopatry and high male-mediated gene flow has likewise been observed in island populations of the endangered Mexican fishing bat (*Myotis vivesi*) in the Gulf of California (Floyd *et al.* 2010). Therefore, the high gene flow I detected between GYE red foxes and conspecifics living at lower elevations does not preclude the GYE population from being a discrete population.

As for the significance of the GYE red fox population, the high elevation alpine environment that it inhabits is certainly an unusual ecological setting, and being the highest known population in North America−it lives year-round at elevations up to and above 3,350 m on the Beartooth Plateau compared to the ESA warranted but precluded Sierra Nevada red fox that lives up to and above 2,775 m on the Sonora Pass in California (Statham *et al.* 2012b)−this is also a unique ecological setting. Little is known about the current distribution of the Rocky Mountain subspecies *V.v. macroura* outside of the GYE, so the existence of other populations of this historically widespread subspecies and the level of connectivity between the GYE and other populations can not be determined. But the concentration of rare cytochrome *b* A3 haplotypes found in the GYE, compared to the diversity of derivatives of the A haplotype found across the western U.S. (Volkmann *et al.* 2015), does suggest that this is a surviving historical population that is currently rare or extirpated from the rest of its historical range. Also the markedly different morphological characteristics of this population, namely the high frequency of light coat colors and hairy feet, may also be significant distinctions, especially if they have a genetic basis as suspected from the F_{ST} outlier microsatellite loci that share chromosomes with genes controlling hair color dilution and hair length. Therefore, multiple lines of evidence indicate that the GYE red fox population is a significant population as well as a discrete population.

But the conservation status of the GYE red fox population may be more difficult to determine. Like the Sierra Nevada red fox, this population has likely always occurred in low densities due to resource scarcity in alpine environments (Perrine *et al.* 2010).

Also, much of its range is already in protected areas including national parks and wilderness areas where human activities, including harvest and motorized use, are restricted. Nevertheless, potential threats and the significance of their impacts may be similar to those described in the ESA listing decision for the Sierra Nevada red fox (USFWS 2015). These include anthropogenic threats like mortalities from vehicle collisions, hunting and trapping, domestic dog attacks, rodenticide consumption, and disturbance from snowmobiles and other recreational activities on and near the Beartooth Scenic Highway, as well as climate-related threats like the expansion of coyotes, wolves, and non-native foxes to higher elevations with declining snowpacks, and the decline of historical food resources like whitebark pine.

Hopefully, these findings will inspire state wildlife management agencies to recognize red foxes in the GYE as a native species–the red fox currently receives no protection in either Montana or Wyoming outside of national parks–and monitor the population and potential threats against it before ESA protection becomes necessary. That way, the region's economic and recreational resources may be preserved along with its unique natural resources like this red fox population.

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