DISTRIBUTION OF NATIVE AND NONNATIVE ANCESTRY IN RED FOXES ALONG AN ELEVATIONAL GRADIENT IN CENTRAL COLORADO

A Thesis

by

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ABSTRACT

The red foxes (*Vulpes vulpes*) indigenous to the mountains of the western United States are high-elevation specialists that could face range reduction due to climatic warming, as well as potential encroachment, loss of adaptive alleles, and displacement by introduced nonnative red foxes. I investigated the genetic integrity of the native Rocky Mountain red fox (*V. v. macroura*) in Colorado, through analysis of the composition, distribution, and patterns of gene flow between native and nonnative red fox populations along an elevational gradient. The study area spanned the high plains around Denver in the east to the alpine zone of the Rocky Mountains adjacent to Gunnison and Crested Butte in the west. I used microsatellite and mitochondrial DNA (mtDNA) from Colorado foxes, along with previously published reference data from other native western and nonnative populations, to evaluate the distribution of native versus nonnative ancestry and its relationship to elevation, distance, and landscape-type. Nonnative red fox ancestry predominated in Denver and low-lying areas, whereas native ancestry was most prevalent at high elevations. The genetic integrity of foxes at higher elevations (i.e., within the historical native range) was greater in terms of mtDNA than nuclear DNA, consistent with higher male-mediated gene flow. At high elevations, nonnative admixture was most pronounced in human-altered landscapes. My findings provide baseline data necessary to monitor future trends of these Rocky Mountain populations and serve as foundations for proactive management of the two endangered mountain red fox subspecies to the west.

DEDICATION

I dedicate this research and path to those who love me, those who support me, and all of my students, who *all* inspire me.

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In the end, I am one lucky girl who will continue to pay it forward.

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INTRODUCTION

As a species, the red fox (*Vulpes vulpes*) can be characterized as a highly adaptive habitat generalist and the world's most widely distributed terrestrial carnivore (Lariviere and Pasitschniak-Arts 1996). However, Afro-Eurasian and North American red foxes reflect two deeply divergent lineages, which reflect distinct evolutionary histories and, most likely, ecologies (Statham et al. 2014). Further, within North America, red foxes were isolated in three refugial groups during the late Pleistocene, also corresponding to distinct evolutionary trajectories (Aubry et al. 2009). The three refugial groups correspond approximately to contemporary Alaskan, eastern Canadian, and western United States (US) regions. The red foxes of the western US collectively represent an ecologically and genetically distinct red fox lineage that includes four currently recognized subspecies (Aubry et al. 2009; Sacks et al. 2010): the Cascades red fox (*V. v. cascadensis*) of Washington, the Sierra Nevada red fox (*V. v. necator*) of Oregon and California, the Rocky Mountain red fox (*V. v. macroura*) of several Rocky Mountain and Great Basin states, and the Sacramento Valley red fox (*V. v. patwin*) of California. Except for the Sacramento Valley red fox, these western red foxes appear to be ecologically specialized to high elevations (Aubry et al. 2009). The distinctiveness of these mountain red fox subspecies is supported by morphological characters including smaller body size and larger surface area (composed largely of hair) on soles of feet thought to reduce foot loading for adaptation to travel on snow (Roest 1977; Aubry 1983; Fuhrman 2002).

Mountain red foxes as a whole appear to have undergone a range reduction (Sacks et al. 2010). As with other organisms restricted by specialized adaptation to high elevations, mountain red foxes increasingly face elevational shifts in vegetation, asynchronous availability of food and habitat resources, and potential for decreases in range corresponding to climate change (Inouye et al. 2000; Perrine et al. 2010). Additionally, low elevation species, including larger competitors such as the coyote (*Canis latrans*) or gray fox (*Urocyon cinereoargenteus*), can shift up-slope and encroach

upon, compete with, and prey upon naïve animals (Van Etten et al. 2007; Perrine et al. 2010). Another more insidious threat comes from nonnative red foxes, which originated from twentieth century fur farms (Statham et al. 2011, 2012a; Sacks et al. 2016). Although these fur-farm foxes ultimately were North American in origin (bred originally from wild-caught eastern Canadian and Alaskan foxes), they presumably lack specialized adaptations of mountain red foxes to the high-elevation environment and reflect multiple generations of selection for a captive environment (Balcom 1916; Laut 1921; Statham et al. 2011). Nonnative red foxes can potentially impact native red foxes through competition, genetic admixture, or genetic swamping and loss of locally adaptive alleles (Sacks et al. 2011; Statham et al. 2011, 2012a).

In contemporary Colorado, red foxes occur continuously from 1,600 m elevations to above 4,200 m, yet historically they were rarely observed below 2400 m (Warren 1910). Genetic analysis of a few modern specimens from high-elevation portions of the Rockies in comparison to historical museum specimens confirmed the continued presence of native Rocky Mountain red fox at high elevations (Aubry et al. 2009; Sacks et al. 2010). However, the genetic composition of red foxes from low to intermediate elevations of the Rocky Mountains and adjacent lowland plains is unknown (Armstrong 2011). One hypothesis is that these low-elevation foxes originated from westward expansion of red foxes from the southeastern USA (Kamler and Ballard 2002), which include native eastern, fur-farm, and potentially European sources (Kasprowicz et al. 2016).

Alternatively, these foxes could derive from local fox farms. Fox-farming in Colorado began in 1922 using breeding pairs imported from Southeastern Canada (Norman 2008). In the 1950s, some of these farmed red foxes reportedly were released directly into the Denver area due to a decrease in demand for fur (Norman 2008). Lastly, it is possible that the red foxes at lower elevations originate from recent downslope expansion of native Rocky Mountain red foxes, as was suggested in more northerly locations of the Rocky Mountains (Fichter and Williams 1967). Because fur-farm-derived nonnative red foxes are essentially feral and tend to be associated with urban and

agricultural landscapes (Lewis et al. 1999; Statham et al. 2012a; Kasprowicz et al. 2016; Sacks et al. 2016), I hypothesized that those in and near Denver on the western edge of the High Plains (where croplands are extensive; Chapman et al. 2006) reflect nonnative ancestry.

If indeed low-elevation red foxes stem from westward-expanding or translocated populations, then it is important to determine the genetic relationship between these and Rocky Mountain red foxes. Elsewhere, native and nonnative red fox populations interbred only within a narrow hybrid zone beyond which they maintained genetic distinctiveness, potentially through reproductive barriers and competitive exclusion (Sacks et al. 2011). Limited genetic and morphological evidence similarly suggested the presence of a hybrid zone between a high-elevation native and low-elevation nonnative red fox population in the northern Rocky Mountains (Fuhrman 2002; Swanson et al. 2005). Alternatively, foxes could potentially blend seamlessly into a hybrid swarm (Rhymer and Simberloff 1996), as has been observed in other canids under certain circumstances (e.g., Fain et al. 2010).

OBJECTIVE

My objective was to better understand the composition, distribution, and gene flow among red fox populations in Colorado along an elevational gradient spanning Denver in the east to Gunnison and Crested Butte in the west. I evaluated the predictions that native ancestry dominated at higher elevations, particularly alpine and subalpine climate-zones, more westerly locations, and unaltered landscapes and, conversely, that nonnative ancestry dominated lower elevations, easterly locations, and more urban and agricultural (rural) landscapes. I also investigated the degree to which native and nonnative populations interbred, in particular, whether admixture was limited to narrow contact zones or was relatively widespread.

STUDY AREA

The study area in central Colorado was 240 km by 80 km (19,200 km^2) and extended from Denver (39.737567°N, 104.9847179°E) in the east to Crested Butte (38.8697146°N, 106.987823°E) and Gunnison (38.544444°N, 106.928333°E) to the west. The city of Denver is located at the interface of the Front Range mountain chain to the west and the high plains to the east. The Front Range-Rocky Mountain interface is considered part of the Southern Rocky Mountain Ecoregion (Bailey et al. 1994; Chapman et al. 2006) and is characterized by a steep elevation gradient in which the elevation changes from approximately 1,600 m and exceeds 3,100 m over a distance approximately 60 km due west of Denver. The area is characterized by of a mix of alpine and subalpine meadows, aspen (*Populus tremuloides*) stands and subalpine fir (*Abies spp*.), and an undulating elevation range of 1,520 m to 4,328 m. The southwestern portion of the study area, the Gunnison Basin, was dominated by sagebrush (*Artemesia* spp) steppe and was believed not to have supported red foxes historically (P. Magee, Western State Colorado University, personal communication).

COLLECTION METHODS

Scats were collected and stored in 15 mL centrifuge tubes with 12 mL of silica desiccant (Janečka 2008). I sampled scats during two 6-week field seasons in June and July of 2012 and 2013 from city and town neighborhoods and approximately 30 km of randomly selected public trails each in both rural and natural areas that were at least 5 km from town centers. Samples were categorized as "urban" if they were located within city or town limits, a landscape that dominated by infrastructure and multi-storied buildings. Some of these samples were collected from wildlife rehabilitation centers that housed red foxes documented to have been found in the Denver city limits. Samples were considered "rural" if they were they were discovered in a setting outside of town or city limits with the landscape heavily influenced by human structures, such as agricultural areas. Samples were categorized as "natural" if they were found in areas without human structures present (primarily in National Forest lands in alpine and subalpine zones). I located additional scats with the aid of citizen scientists. Specifically, I developed a phone and web-based red fox reporting system (http://IFoundaFox.org) to collect locational data on potential areas to search for red fox genetic material. I advertised the website via fliers distributed across the study area in public posting areas, such as coffee houses and community centers, and through environmentally based organizations (e.g., The Denver Audubon Society), government agencies (e.g., U. S. Fish and Wildlife Service), and social media. I allocated search effort according to apparent validity of reports and interviews as well as to ensure a representative sample of the study area.

Samples

I collected 184 scats from the field and used these along with 10 tissue samples from foxes trapped near Gunnison, Colorado provided by Dr. Patrick Magee of Western State College, and, for reference, 6 tissue samples from foxes in Salt Lake City, Utah carrying nonnative mtDNA haplotypes (B.N. Sacks, unpublished data). Although samples were distributed continuously to capture ranges of elevation, longitudes, and

urban/rural/natural habitats, I nevertheless partitioned the study area into 5 sampling "sites" based on proximity and landscape features to facilitate tests of Hardy-Weinberg and gametic (linkage) equilibrium and for descriptive purposes: Crested Butte (CB), Gunnison (GU), Leadville (LV), Evergreen (EVG), and Denver. For reference, I used the 6 Utah samples and 91 previously published microsatellite genotypes (Sacks et al. 2010; Statham et al. 2012a), representing historical museum specimens from the Sierra Nevada (n = 18) and Rocky Mountains (n = 11), modern specimens from the northern Rocky Mountains (n = 28) and the Sierra Nevada (n = 3), as well as nonnative populations in California (n = 31).

LABORATORY PROCEDURES

I extracted DNA at Texas A&M University, College Station using Qiagen Stool DNA extraction kit and manufacture prescribed protocols (Qiagen, Valencia, California). To ensure compatibility of genotypes in this study with the previously published reference genotypes, I conducted all microsatellite genotyping and mitochondrial DNA (mtDNA) sequencing at the University of California, Davis, where the previous work had been conducted. I genotyped each sample three times (i.e., 3 independent polymerase chain reactions [PCR]) at 14 microsatellite loci, AHT133, AHT140, c01.424PET, FH2004, FH2010, FH2088, FH 2289, FH2328, FH2380, RF08.618, RF2001, RF2054, RF2457, RFCPH2, as previously described (Moore et al. 2010; Sacks et al. 2010). I electrophoresed PCR products on an ABI 3730 capillary sequencer (Applied Biosystems, Foster City, California), and assessed allele sizes relative to a fragment size standard, Genescan 500 LIZ (Applied Biosystems), using genotyping software STRand (Toonen and Hughes 2001). Alleles were binned along with reference genotypes (Sacks et al. 2010; Statham et al. 2012b).

I sequenced 354 base pairs (bp) of the cytochrome *b* gene and 342 bp of the D-loop gene for direct haplotype comparisons to prior North American red fox studies using previously described laboratory methods for PCR amplification, chemistry, and cycle conditioning. Specifically, I used the primer pair RF14724 and RF15149 to amplify the cytochrome *b* fragment (Perrine et al. 2007) and the primer pair VVDL1 and VVDL6 to amplify the D-Loop fragment (Aubry et al. 2009). I purified PCR product using ExoSap-IT (Affymetrix, Inc., Santa Clara, California) and sequenced in both forward and reverse directions using the ABI BigDye Terminator cycle sequencing kit 2.0 (Applied Biosystems, Inc.). I visually aligned sequences using Sequencher 4.5 software (Gene Codes, Inc., Ann Arbor, Michigan). Because the cytochrome *b* and D-loop markers were linked, I concatenated them into 696 bp composite haplotypes, enabling direct comparison to previous studies, which have identified native and nonnative lineages (e.g., Aubry et al. 2009; Statham et al. 2012a; Sacks et al. 2016).

DATA ANALYSIS

Microsatellites

I estimated probability of allelic dropout and false alleles for fecal genotypes by comparing each replicate of each sample to its final consensus genotype. The per-replicate probability of genotyping error was estimated as the ratio of total errors to number of replicates corresponding to heterozygous consensus genotypes (Bonin et al. 2004). I used Microsatellite Toolkit for Microsoft Excel (Park 2001) to detect matching genotypes among scat samples (i.e., multiple samples from the same individual) and to estimate observed heterozygosity (H_O), expected heterozygosity (H_E), and average numbers of alleles. To allow for genotyping error, I conservatively considered two genotypes to come from the same individual if they shared >85% of their alleles and all of their mismatches were consistent with allelic dropout (Sacks et al. 2011). I assessed Hardy-Weinberg equilibrium and linkage (gametic) disequilibrium using Genepop 4.3 (Rousset 2008) for the five above-defined sites. I used a sequential Bonferroni correction method to adjust statistical significance levels for multiple comparisons (Rice 1989).

Mitochondrial DNA

I first identified the species of scat-derived sequences through a Basic Local Alignment Search Tool (BLAST; Altschul 1990) search of the Nucleotide database in GenBank, using 98% homology with my cytochrome *b* sequence as my criterion for species-typing. Because numerous studies have thoroughly described the mtDNA composition of native and nonnative western populations and fur-farm stock (e.g., Aubry et al. 2009; Statham et al. 2011, 2012a; Kasprowicz et al. 2016; Sacks et al. 2016), I could directly investigate the native/nonnative maternal-line composition of sampling sites.

Nuclear Genetic Admixture

To assess the degree of native versus nonnative ancestry composing nuclear genomes of red foxes, I analyzed microsatellite data using a Bayesian Markov Chain Monte Carlo (MCMC) multi-locus approach in program STRUCTURE v 2.3.4

(Pritchard et al. 2000). Because my interest was specifically in native and nonnative ancestry, I classified ancestry in terms of two discrete genetic clusters (i.e., $K = 2$). Preliminary analyses assuming greater numbers of genetic clusters ($K = 3$–8) and estimating the posterior probabilities for each K (and associated statistics; Evanno et al. 2005; Earl and vonHoldt 2012) provided no additional insight (Table 1). I conducted analyses both using "no prior information" and with "prior information;" in the latter case, I used as "knowns" the previously published historical native and modern nonnative samples as a basis for assigning "unknowns." To be conservative and provide a control, I treated as "unknowns" the modern reference samples determined to be native in previous studies (i.e., along with the samples collected in the present study; Sacks et al. 2010; Statham et al. 2012b). I used the admixture model with correlated allele frequencies (Falush et al. 2003) and conducted runs of 1,050,000 MCMC cycles, discarding the first 50,000 cycles as burn-in. For descriptive purposes and logistic regressions (see below), I somewhat arbitrarily denoted individuals as "pure" native or nonnative when their estimated native ancestry fraction (q) was ≥ 0.85 or ≤ 0.15, respectively. Correspondingly, I considered individuals admixed when $0.15 < q < 0.85$.

Isolation by Distance

I assessed the presence of genetic isolation-by-distance for microsatellite and mitochondrial DNA using Mantel tests performed in Arlequin 3.5 (Guo and Thomson 1992; Excoffier and Lischer 2010) and partial Mantel tests using program PASSaGE (Rosenberg and Anderson 2011). I used as my measure of genetic distance linearized F_{ST} estimates [i.e., $F_{ST}/(1-F_{ST})$] computed in Arlequin (Smouse et al. 1986; Slatkin 1995) and tested genetic distance against both Euclidean geographic distance (km) and elevational distance (m).

Correlates of Ancestry

I examined univariate relationships between native mtDNA and nuclear ancestry with elevation, distance west of Denver, and landscape association. I then statistically assessed these relationships in terms of native versus nonnative mtDNA haplotypes and for native/nonnative/admixed microsatellite genotypes using logistic regression. I

performed logistic regressions using SAS statistical software (SAS 2015). Models were evaluated based on Akaike information criterion (AIC; Burnham and Anderson 2004) to select the best predictive models for native and nonnative red fox distribution.

Additionally, to make full use of the continuous nature of my estimates of native nuclear ancestry (i.e., *q*), I conducted an additional set of analyses using %native ancestry (*q*) from the STRUCTURE analysis with no prior information as the dependent variable. To normalize data for correlations and general linear models, I transformed *q* as the arcsin of its square-root (Zar 1996). I then conducted a general linear model in Systat (v9.0; SPSS Inc, Chicago, Illinois) using transformed % native *q* as the dependent variable and elevation, distance west of Denver, and landscape type as independent variables.

RESULTS

I attempted to extract DNA from 140 fecal samples collected in Colorado during 2012 and 2013 summer field seasons along with 10 tissue samples. Based on cytochrome *b* sequencing, 117 of these samples (107 scats, 10 tissue) had usable red fox DNA, whereas 21 scat samples originated from non-target species, and 12 failed to produce usable sequences. The 21 non-target haplotypes were identified through a BLAST search as domestic dog (n = 3), gray fox (n = 2), coyote (n = 14), porcupine (*Erithizon dorsatum,* n = 1), and striped skunk (*Mephitis mephitis,* n = 1).

Microsatellites

I successfully genotyped microsatellites from 77 samples, including all 10 tissue samples, 65 of the 107 fecal samples that had been verified to be red fox based on cytochrome *b*, and 2 of the 12 fecal samples that failed the sequencing attempt. I estimated microsatellite genotyping error for fecal samples based on 60 triplicated 14-locus genotypes to be 12.6% per replicate for allelic drop out and 1.8% per replicate for false alleles. The expected genotyping error in consensus genotypes (i.e., after 3-fold replication) was <1%. Based on allele matching, 10 individual red foxes were represented 29 times in the dataset. After removing the 19 redundant samples, I retained a single microsatellite genotype from each of 58 individuals. Although sample sizes were small in each site, CB and LV were nevertheless significantly out of Hardy Weinberg equilibrium (Table 1), consistent with admixture. Regarding individual loci, FH2088 was significantly out of Hardy Weinberg equilibrium in CB, LV, and GU, as were two other loci (FH2004, FH2328) in CB only. Significant linkage disequilibrium was observed only in Denver (FH2010/FH2054) and CB (AHT140/RF2457, AHT140/C01-424, FH2004/RFCPH2).

mtDNA Ancestry

After removing from the red fox sequence data set 19 mitochondrial cytochrome *b* haplotypes corresponding to multiple sampling of the same individuals, I retained 98 haplotypes for analysis, all of which could be identified as native or nonnative

haplotypes, which were heterogeneously distributed (Fig. 1a). All red fox haplotypes were of North American origin (i.e., no European haplotypes), but included both native and fur-farm haplotypes. In general, native haplotypes occurred in the mountains whereas nonnative haplotypes were primarily distributed in plains or on the margins of mountain ranges.

I successfully sequenced 95 of these samples at the D-loop fragment as well, yielding 7 native and 3 nonnative concatenated haplotypes. I identified 2 novel D-loop sequences, both of which were associated with the native cytochrome *b* haplotype A (and clustered in the "mountain subclade;" Aubry et al. 2009). One of these haplotypes (A-271) was found exclusively in GU and the other novel haplotype (A-270) was dispersed throughout the study area. I deposited the novel sequences in GenBank (Accession Nos. KX766407– KX766408). In general, native haplotypes were more widely dispersed across the study area, whereas the two dominant nonnative haplotypes each were relatively localized, e.g., G-38 to the Denver area and F-17 to the LV area (Fig. 2). Two notable exceptions were putative native haplotypes, A-68, which was concentrated in the Denver area, and A-271, which was concentrated in the GU area.

Nuclear Ancestry

Both admixture analyses (no prior information, prior information) in STRUCTURE indicated considerable contributions of both native and nonnative ancestry to the Colorado samples (Fig. 3). Using no prior information, the average proportional contribution (q) of native ancestry was 74% in CB, 53% in LV, 28% in GU, and 9% in both EVG and Denver. Similarly, using prior information, the average proportional contribution (q) of native ancestry was 75% in CB, 55% in LV, 27% in GU, and 13% in both EVG and Denver. The modern reference samples included from the northern Rocky Mountains and Sierra Nevada assigned primarily to historical native western ancestry as shown previously (Sacks et al. 2010; Statham et al. 2012b), emphasizing the contrast with samples from the southern Rocky Mountains of Colorado in the present study. For all subsequent analyses, I used the estimated ancestry (q) from the model with no prior information.

Isolation by Distance

I did not detect any significant genetic isolation by geographic or elevational distance for mtDNA (Mantel $r = 0.003$, -0.02, respectively, $P > 0.2$), microsatellites (Mantel $r = -0.00$, -0.05, respectively, $P > 0.7$), or by geographic and elevational distance combined using partial Mantel tests for microsatellites or mtDNA ($P > 0.25$).

Landscape Analysis

Univariate relationships of both native maternal ancestry (mtDNA) and native nuclear ancestry (microsatellite) with elevation indicated positive trends, although relationships appeared nonlinear (Fig. 4). Frequency of native mtDNA haplotypes and native nuclear genotypes also tended to be higher in natural than rural and urban landscapes (Fig. 5) and in more westward locations (data not shown). Although the Denver area was most urban, lowest-elevation, and furthest east, the three predictor variables otherwise varied approximately independently of one another (i.e., had low intercorrelation), enabling modeling of the effects on ancestry of these three variables in combination (Fig. 6).

The best model according to the mtDNA logistic regression analyses included all three of these predictor variables (i.e., elevation, distance west of Denver, and landscape type). All models <7 ΔAIC units of the best model included distance west from Denver. The best model according to the microsatellite logistic regression included distance west of Denver and elevation, although also within 2 ΔAIC units of this model were univariate models including elevation, distance to Denver, and one including distance to Denver and Landscape type.

The univariate correlations of native nuclear ancestry (q) with elevation and distance west of Denver were similar ($r = 0.59$, 0.55, respectively, $P < 0.001$). The relationship of % native ancestry (q) with landscape type was marginally significant (1-way ANOVA $F_{2,55} = 2.97$, $P = 0.06$). When all three variables were considered in concert in a general linear model, only elevation was significant ($F_{1,53} = 6.25$, $P = 0.016$); distance to west of Denver ($F_{1,53} = 3.25$, $P = 0.077$) and landscape type ($F_{2,53} = 1.19$, $P = 0.31$) were not statistically significant.

DISCUSSION

I sought to determine the origins of the red fox population in and around the Denver area (east end of my study area) and other locations on the margins of the southern Rocky Mountains and, if nonnative, to assess the degree of population mixing between these populations and the historically native populations of the higher elevation Rocky Mountains to the west and north. Whereas the nuclear genetic data were most informative for estimating relative native and nonnative components of total genomic ancestry, the mitochondrial data provided the most concrete evidence of ultimate origins.

Denver and Low-elevation Populations

My cumulative findings indicated that red foxes in the Denver and other low-lying areas were primarily nonnative, but also contained some native Rocky Mountain red fox ancestry. In particular, nonnative haplotypes dominated in Denver, the adjacent Front Ranges, and along the southern fringes of the "Leadville" segment of the Rocky Mountains. Moreover, these low-elevation areas were primarily nonnative according to nuclear genetic assignments relative to known native and nonnative reference populations. However, I also observed native mitochondrial haplotypes in Denver (approximately 1/3) and in Gunnison (all), even though their nuclear ancestry was assigned primarily as nonnative (91%, 73%, respectively). In both of these cases, only a single native haplotype was found, consistent with introgression from as few as a single native female.

The native haplotype in the Denver area, A-68, was found previously in a single sample collected in 1903 from in the southern extent of the Rocky Mountains of New Mexico (Aubry et al. 2009; Sacks et al. 2010). I did not find this haplotype anywhere outside of the Denver area in the present study, suggesting it was rare or nonexistent in the immediately adjacent Rocky Mountains, which otherwise shared multiple (other) native haplotypes. Therefore, this localized native haplotype could reflect chance introgression of a rare native haplotype via natural means or, alternatively, integration into a local fur farm of translocated native foxes from further south.

Gunnison Population

On the other hand, natural introgression seems more likely in the case of the Gunnison population, which also was found to be primarily nonnative based on nuclear ancestry. Similar to the Denver population, the Gunnison population also carried a native haplotype (exclusively, in fact) that was not widespread across the study area. However, all but two of the samples bundled as "GU" were collected in the urban habitat within the sagebrush steppe in the town of Gunnison, whereas two individuals also carrying this haplotype were sampled from a natural area of montane forest east and upslope of the town of Gunnison. Importantly, the one of these two montane samples that was successfully genotyped also assigned as pure native, providing support for the natural origin of this haplotype in the population despite its relatively limited geographic distribution.

Fox Fur Farm Origins

Another question of interest in this study was whether the nonnative red fox ancestry dominating at the lower elevations arose from local fur farms or population expansion from the east, as had been previously hypothesized (Kamler and Ballard 2002). My results seem more concordant with the former hypothesis, that the nonnative red foxes in Denver and other low-lying areas were sourced from a small number of local farms. Most generally, the dominance of only two maternal fur-farm haplotypes (G-38, F-17) throughout my study area suggests the nonnative component of this population arose from a small number of founders (at least the female component), consistent with escape or release from a small number of fur farms. Second, the geographic distribution of G-38 and F-17 suggested distinct foci corresponding to distinct points of origin. In particular, 24 of the 26 nonnative haplotypes sampled in Denver and the adjacent Front Ranges were G-38, whereas 5 of 8 of the nonnative haplotypes found in scattered locations along the southern margin of the Rocky Mountains (referred to here as the "Leadville" site) were F-17. Multiple other fur-farm and eastern haplotypes occurred in the Midwest, yet apparently did not make it into this population (Statham et al. 2012a). I also observed no European haplotypes in this study,

which would have provided clear evidence of an eastward expansion (Kamler and Ballard 2002), particularly given that European mtDNA haplotypes composed approximately 1/3 of those sampled on the eastern seaboard (Kasprowicz et al. 2016).

In contrast, the localization of nonnative haplotypes observed in this study was much more in line with the pattern described in a previous study of known nonnative populations in California, in which 10 nonnative haplotypes were found in the state, but with no more than 1 or 2 dominant haplotypes at any given location (Sacks et al. 2016). In that case, the interpretation was that female foxes (i.e., which pass on mtDNA) tended to remain close to points of introduction, thereby providing a longer lasting footprint of their origins than nuclear DNA. Thus, my findings suggest that the nonnative sources in Colorado were local, derived from a small number of the many fur farms historically present in the Denver area (Norman 2008), rather than derived from a westward expansion, as previously hypothesized (Kamler and Ballard 2002).

Admixture

My second objective was to better characterize the geography of admixture between the native Rocky Mountain and nonnative introduced populations. In general, I found extensive genetic mixing, particularly with respect to nuclear alleles. Although the distribution of native and nonnative mitochondrial haplotypes appeared consistent with a relatively narrow contact zone running from southwest to northeast along the base of the Rocky Mountains, the nuclear assignments suggest that considerable admixture has occurred on a genomic level (e.g., Fig. 1). Although native ancestry tended to remain higher at higher elevations, I also found considerable admixture in some foxes at the highest elevations sampled; these tended to be areas closest to recreational areas of high human use. Thus, as supported by logistic regressions, both human-impacted habitat and elevation seem to affect the genetic integrity of native foxes. Although I also found models including the distance from Denver to be supported, this seems less likely to be important in light of my finding that nonnative populations appear to have been seeded relatively independently rather than reflecting a westward expansion.

If, as previously hypothesized, montane red foxes are uniquely specialized to climatically extreme high-elevation natural environments (Aubry et al. 2009), they could be in danger of losing such locally adaptive alleles through genetic swamping by nonnative red foxes, particularly if nonnative red foxes are supported by human subsidies. Metabolic responses to elevation and temperature could be key to distinctions between montane and nonnative red foxes. Nonnative red foxes may lack the physiology required to accommodate the high-elevation oxygen demand while also expending the energy necessary for hunting prey and maintaining body core temperatures in extremely cold, snow-packed natural environments (Storz 2007). If so, the encroachment of human-dominated islands of habitat at high elevations could facilitate the expansion of nonnative red foxes alleles into higher elevations. In particular, human-derived resources, such as high energy anthropogenic foods, could provide sufficient energetic supplements to enable otherwise locally maladapted red foxes to thrive (Bateman and Fleming 2012).

On the other hand, thermodynamic adaptations of native foxes advantageous at high elevations could be physiologically detrimental at lower elevations (Monge and Leon-Velarde 1991). For example, high-elevation animals with a hemoglobin variant characterized by greater oxygen affinity might have the physiological challenge of reduced efficiency at lower elevations for off-loading oxygen from red blood cells (Weber 2007). If so, native red fox alleles that increase oxygen-binding capacity could be selected against at lower elevations, potentially accelerating the loss of montane-adaptive alleles.

Evidence of admixture and introgression in the high-elevation regions of the study area is of special concern particularly in light of climate change. Average temperatures in the mountainous regions in Colorado have increased 1.4°C since 1975 (Inouye et al. 2000). Predictions indicate that a 3°C change in this region would shift the lower boreal zone upward in elevation by as much as 500 m, with a concomitant 66% reduction of boreal habitat (Murphy and Weiss 1992). It is possible that climate change could induce significant greening at high elevations, with a positively correlative rodent

response (Ulateig 2010), and therefore a positive red fox population response in native and nonnative red foxes. If nonnative red foxes are not capable of surviving and reproducing in environments that are both natural and high elevation, native red fox may find the increase in food abundance advantageous to their survivorship. Alternatively, if the overall climatic temperature or the ability to exploit snow pack for hunting in the winter is the barrier for nonnative red fox to be successful, warming in the high elevation clines combined with ample food resources may result in further genetic swamping and displacement of the native-type (Rhymer and Simberloff 1996; Guralnick 2007). Continued research is required to determine if the Rocky Mountain populations have the same reproductive barriers, chiefly competitive exclusion, that native and nonnative populations appear to have in other parts of the West (Sacks et al. 2011).

SUMMARY AND CONCLUSION

My findings provide baseline data necessary to monitor future trends in Rocky Mountain red foxes in Colorado as they relate to environmental changes at higher elevations caused by climate change, human encroachment, and admixture with nonnative red fox. Despite their nonnative admixture, Rocky Mountain red foxes remain considerably more numerous and widespread throughout their historical range than the mountain red fox subspecies of the Pacific Crest ranges to the west. Therefore, information gathered on Rocky Mountain red foxes, as in the present study, could bolster the data available to make decisions regarding conservation of the more endangered Sierra Nevada red fox and Cascade red fox (Perrine et al. 2007; Sacks et al. 2010; Statham et al 2012b). Although there is evidence of recent nonnative admixture in Sierra Nevada red foxes (Quinn and Sacks 2014), I am aware of none yet in the Cascade red fox of Washington. Thus, the present study provides a window into one potential future for the other two mountain red fox subspecies. Further research is needed to understand the niche and habitat requirements of the native mountain red fox relative to that of the fur-farm descendants, including responses to climate change and anthropomorphic changes to the landscape. Documenting patterns in space and time to better understand the cause-and-effect relationships could provide opportunities for proactive management of these Colorado populations and other mountain red fox populations.

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APPENDIX

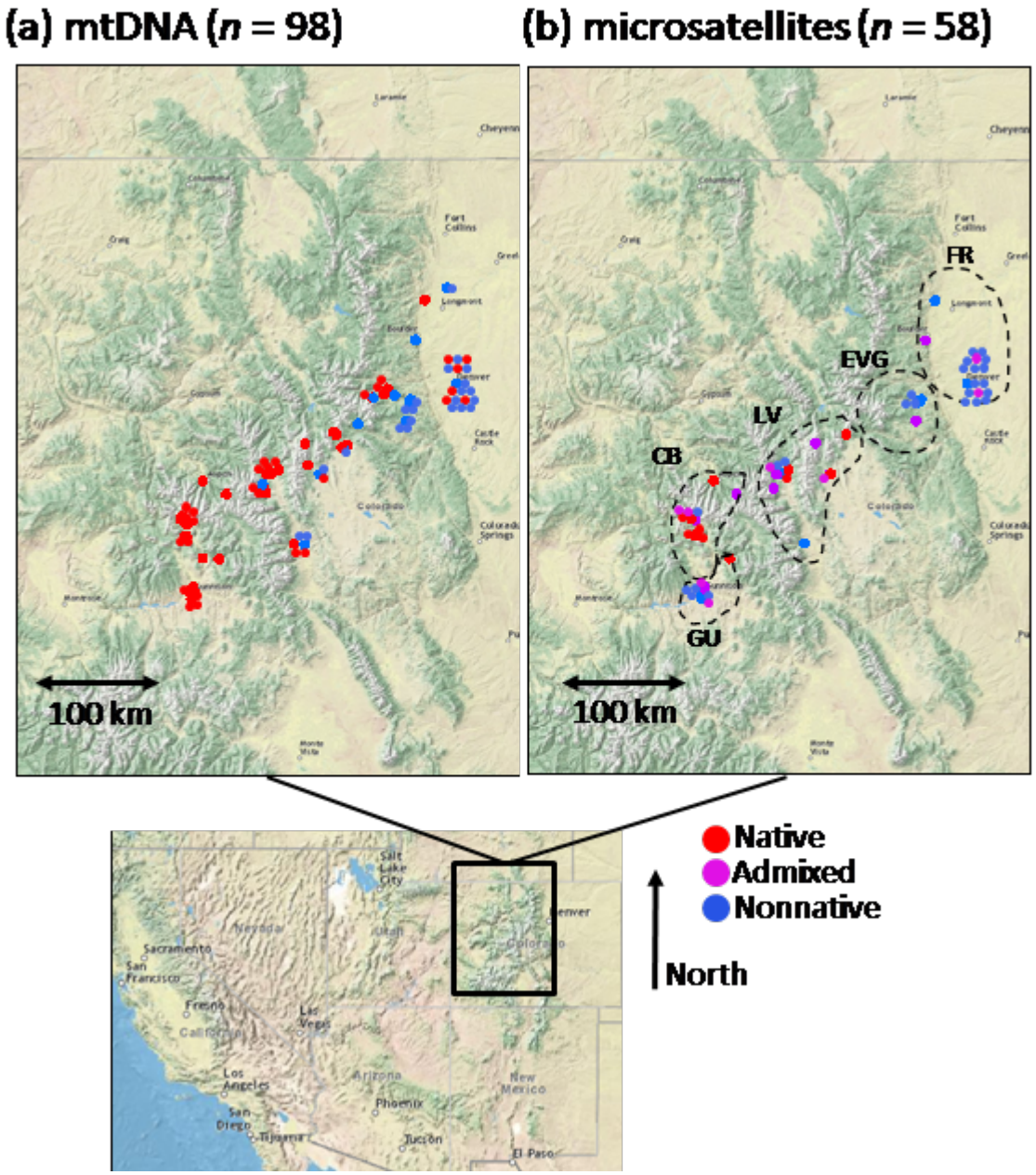

Figure 1. Distribution of red fox genetic samples with respect to (a) native and nonnative mitochondrial haplotypes (n = 98) and (b) native, nonnative, and admixed ($0.15 \geq q \geq 0.85$) nuclear (microsatellite) ancestral assignments based on STRUCTURE analyses with no prior information (n = 58). Note: Nonnative haplotypes refer to those associated with fur-farming, which themselves derive wholly from North American stock; no European haplotypes were found in this study.

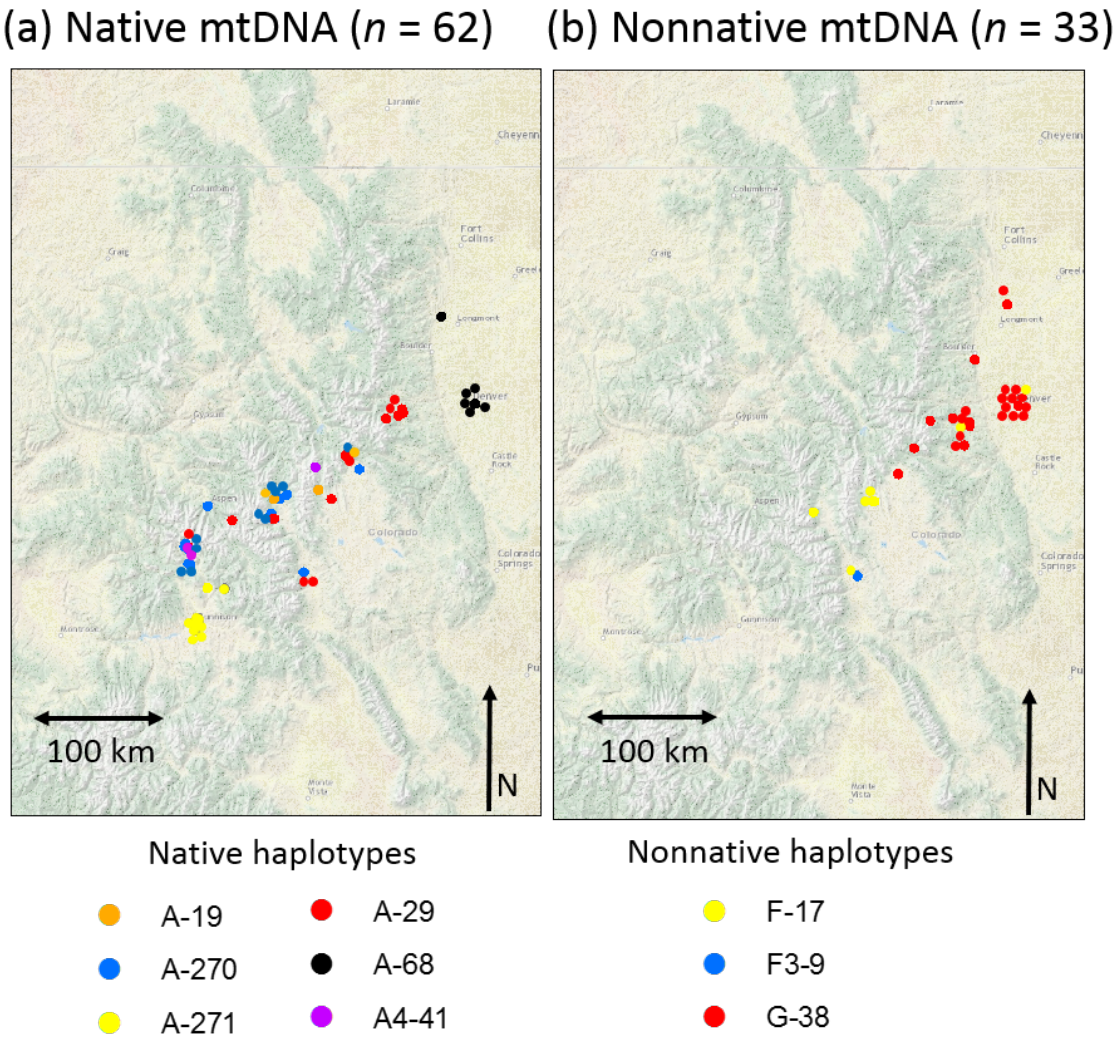

Figure 2. Distribution of native (a) and nonnative (b) mitochondrial haplotypes of red fox in the southern Rocky Mountains of Colorado, illustrating high dispersion and higher elevation of most native haplotypes (except A-68 and A-271) and more localized occurrence of nonnative haplotypes at lower elevations and the Front Ranges. Note: Nonnative haplotypes refer to those associated with fur-farming, which themselves derive wholly from North American stock; no European haplotypes were found in this study.

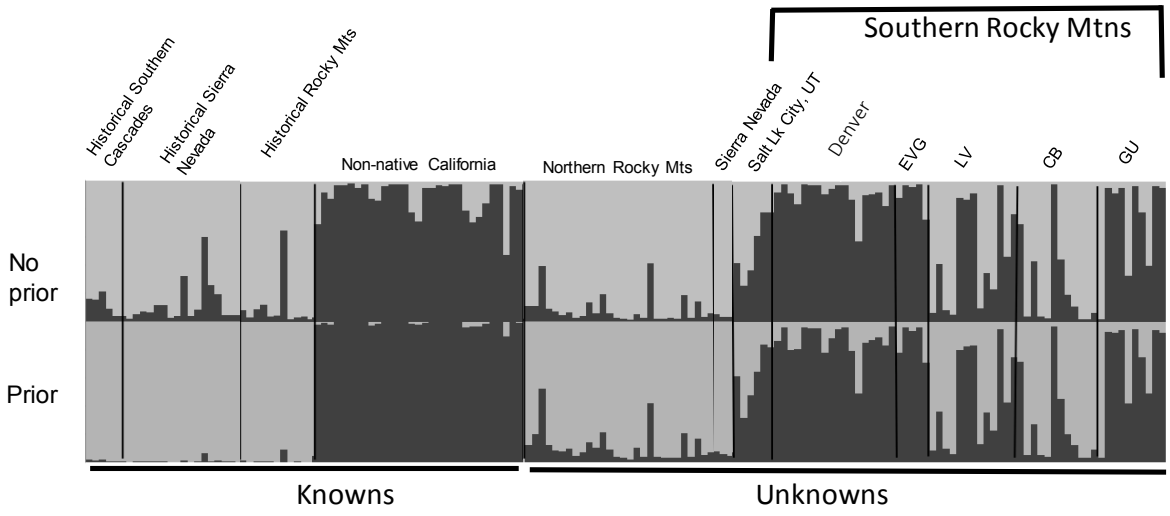

Figure 3. Admixture analysis in program STRUCTURE with no-prior information and using prior information from "knowns" to estimate native/non-native admixture fractions of red foxes from the southern Rocky Mountains of Colorado (n = 58) relative to previously published genotypes of "known" native western United States historical museum specimens and non-native California red foxes, along with modern samples of "unknown" ancestry (n = 97) from the northern Rocky Mountains, Sierra Nevada, and Salt Lake City, Utah (Sacks et al. 2010; Statham et al. 2012b; B. N. Sacks unpublished data), illustrating highly admixed ancestry of the southern Rocky Mountains of Colorado. Previously published samples from the northern Rocky Mountains and Sierra Nevada, which were treated as unknowns in the present analyses, were classified here the same as in previous analyses (Sacks et al. 2010; Statham et al. 2012b).

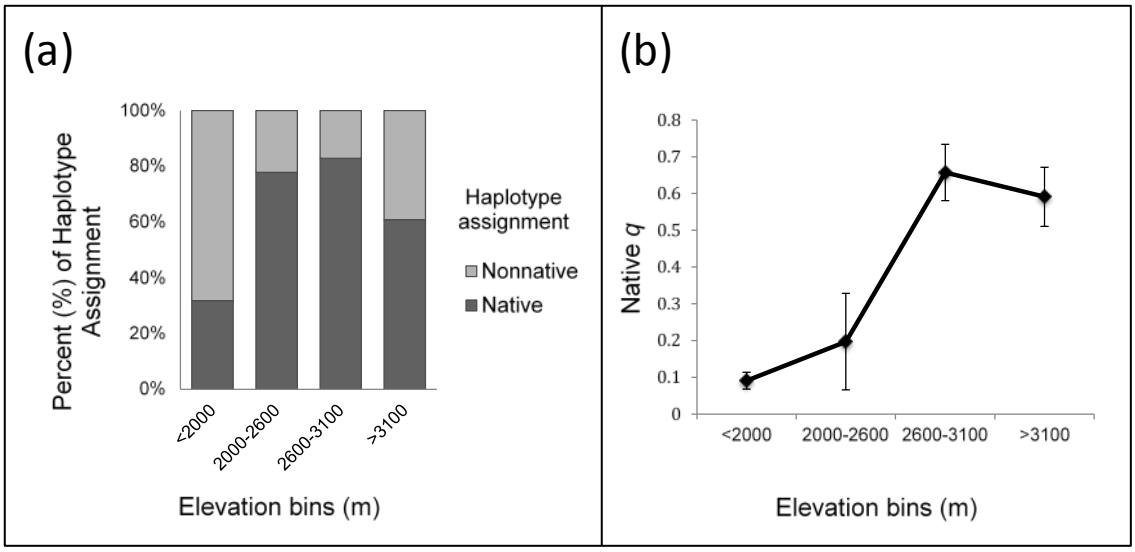

Figure 4. Relationship between elevation versus (a) frequencies of native and nonnative mtDNA haplotypes (n = 98) and (b) ancestral native fraction, q, estimated in STRUCTURE with no prior information (n = 58).

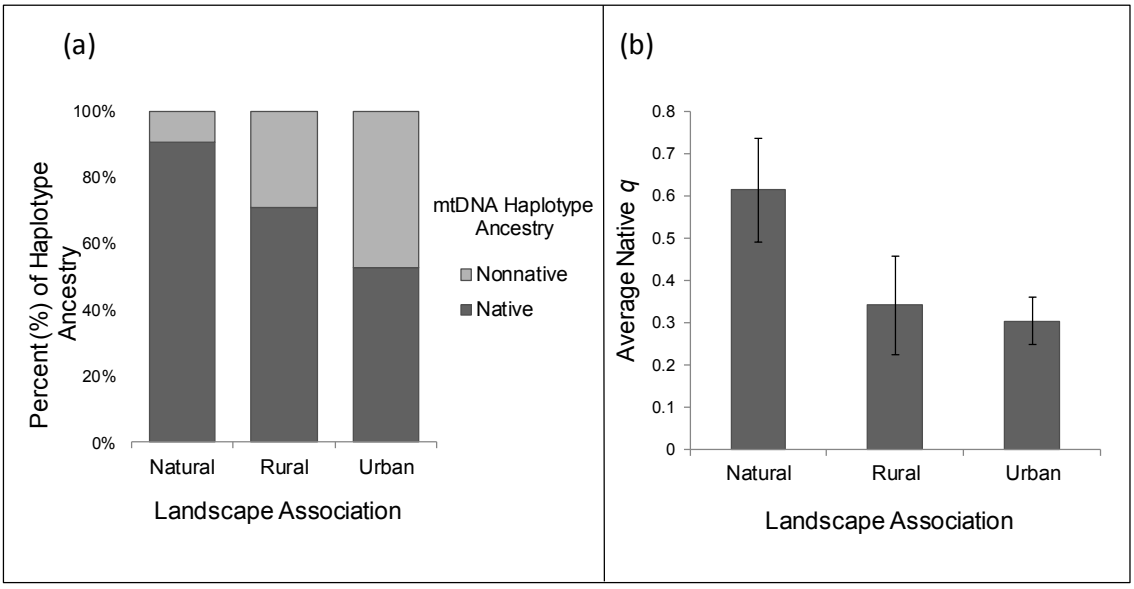

Figure 5. Relationship between landscape association versus (a) frequencies of native and nonnative mtDNA haplotypes (n = 98) and (b) ancestral native fraction, q, estimated in STRUCTURE with no prior information (n = 58).

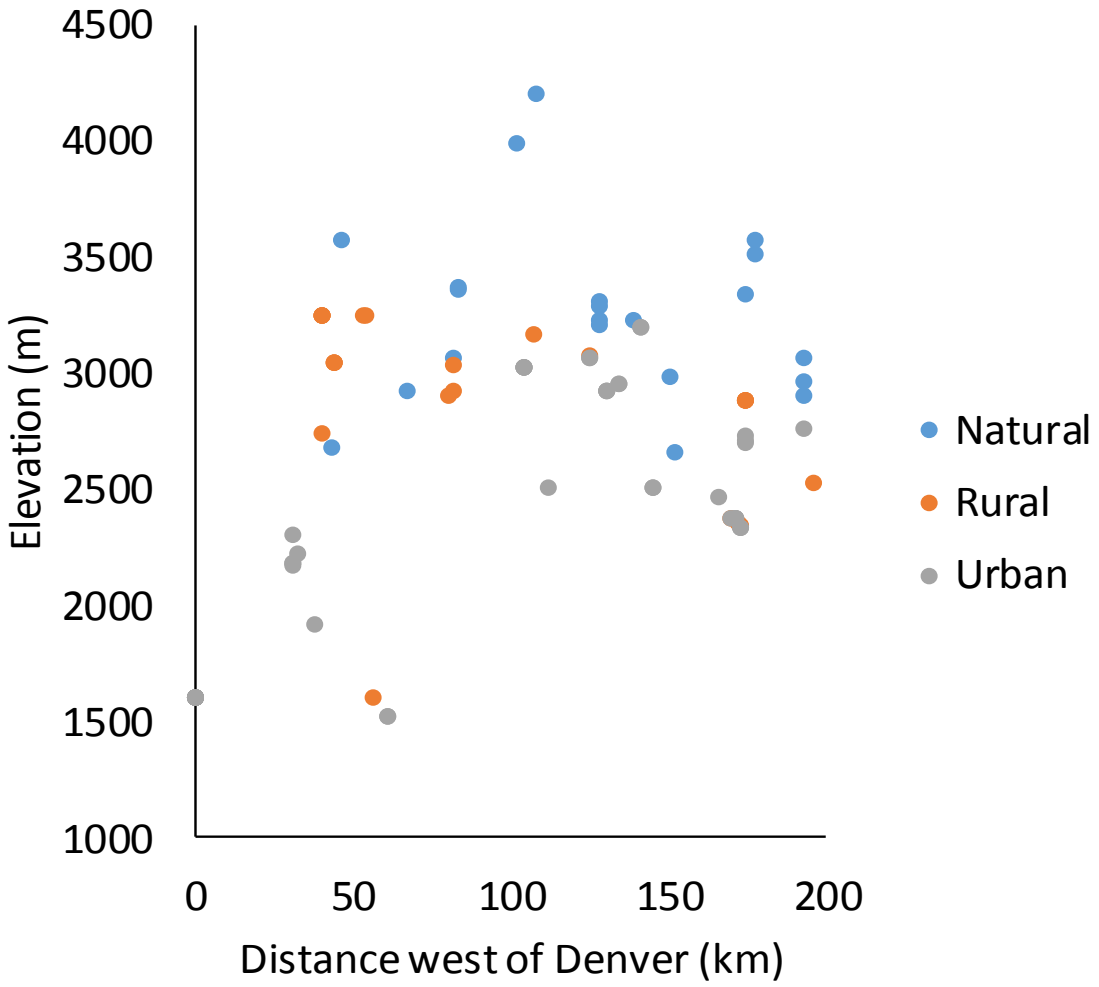

Figure 6. Relationships of elevation to distance-west for 3 landscape types, illustrating independence among predictor variables based on 98 sample locations used in mtDNA analyses (a subset of which was also used in microsatellite analyses).

Data set	K	Avg LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
Total						
	1	-6668.2	1.2	–	–	–
	2	-6432.3	6.3	235.97	81.30	13.0
	3	-6277.6	7.0	154.67	31.60	4.5
	4	-6154.5	18.7	123.07	18.73	1.0
	5	-6050.2	13.9	104.33	65.60	4.7
	6	-6011.5	3.0	38.73	19.97	6.8
	7	-5952.8	21.4	58.70	228.47	10.7
	8	-6122.5	47.6	-169.77	–	–
Colorado						
	1	-1998.9	1.4	–	–	–
	2	-1965.3	22.6	33.57	28.73	1.3
	3	-1903.0	8.7	62.30	14.67	1.7
	4	-1855.4	29.2	47.63	18.23	0.6
	5	-1826.0	13.3	29.40	51.63	3.9
	6	-1848.2	47.1	-22.23	812.63	17.3
	7	-2683.1	1460.9	-834.87	1650.70	1.1
	8	-1867.2	34.7	815.83	–	–

Table 1**.** – Average (Avg) and standard deviations (StDev) of the estimated logarithm (Ln) probability of the data and associated statistics from Evanno et al. (2005) for 8 levels of K using outputs from STRUCTURE runs on the total data (reference plus Colorado data; $n = 97$) and Colorado data only ($n = 58$).