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Natural rewilding of the Great Basin: Genetic consequences of recolonization by black bears (*Ursus americanus*)

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Abstract

Aim: In the mid-20th century, many populations of large-bodied mammals experienced declines throughout North America. Fortunately, within the last several decades, some have begun to rebound and even recolonize extirpated portions of their native range, including black bears (*Ursus americanus*) in the montane areas of the western Great Basin. In this study, we examine genetic variation in source and recolonized areas to better understand the genetic consequences of recolonization.

Location: Western Great Basin, USA.

Methods: Using multiple loci, we characterized genetic variation among source and recently recolonized areas occupied by black bears, tested for population structure and applied approximate Bayesian computation to test competing hypotheses of demographic history. We assessed signals of gene flow using expectations of genetic consequences derived from alternative modes of recolonization (bottleneck, metapopulation, island model) and tested for significant signals of genetic bottlenecks in areas recently recolonized by black bears.

Results: As anticipated from field survey data and hypothesized expectations, genetic variation of western Great Basin black bears retain an overall signature of demographic decline followed by recent rebound. Furthermore, results reveal that bears in the recolonized range are minimally differentiated from the source area, but newly established subpopulations have lower effective population sizes and reduced allelic diversity. Nevertheless, recolonized areas fail to show a significant signal of a genetic bottleneck. Moreover, bears occupying recolonized areas experience asymmetric gene flow, yielding strong support for a model of genetic connectivity that is best described as a metapopulation.

Main Conclusion: This study presents one of the few empirical examples of genetic consequences of natural recolonization in large-bodied mammals. Furthermore, these results have implications for understanding the complexities associated with the genetic consequences of recent and ongoing recolonization and highlight the need to develop management strategies uniquely tailored to support connectivity between source and recolonized areas.

KEYWORDS

BIMr, black bear, DIYABC, metapopulation, PCoA, Sky Island

1 | INTRODUCTION

Many species face intensifying extinction risk, at least in a portion of their range, because of increasing urbanization and habitat loss (Lambers, 2015; Thomas et al., 2004; Urban, 2015), and because climate envelopes are shifting on the landscape (Guisan, Petitpierre, Broennimann, Daehler, & Kueffer, 2014). Because of these changes, leading-edge populations are expected to opportunistically occupy new areas, but certain populations will likely be left dwindling at the trailing edge of suitable conditions and may go locally extinct. While we have a general understanding of the genetic consequences of geographically widespread and relatively long-term (re)colonizations, such as those that characterize expansion into previously glaciated parts of the Northern Hemisphere (Excoffier, Foll, & Petit, 2009; Hewitt, 2000; Lessa, Cook, & Patton, 2003), we know comparatively little of the genetic consequences of very recent or perhaps ongoing natural recolonizations. Understanding the fine-scale process or mode of recolonization and its genetic consequences is of broad interest in ecology and evolution and imperative to successful restoration and climate change planning.

While there are few empirical examples of genetic consequences of fine-scale recolonizations, alternative scenarios predict dramatic differences. For example, genetic differentiation can increase because of founder effects associated with few colonizing individuals (Templeton, 1980; Wright, 1940) that fail to represent total variation in the source population, and because of subsequent isolation and lack of continued gene flow. Such a model of recolonization would result in a genetic bottleneck in the recolonizing population(s). In contrast, if newly colonized areas experience high connectivity and freely exchange migrants with other areas (i.e., island model) (Slatkin, 1987; Wakeley & Aliacar, 2001), then gene flow would be expected to prevent genetic differentiation. These two classic models represent somewhat idealized extremes, while a metapopulation model captures perhaps a more realistic alternative where connectivity is maintained between source and colonized localities, but that connectivity (gene flow) is asymmetric (Table 1). Empirical genetic datasets coupled with powerful new simulation approaches allow alternative scenarios such as these to be directly compared and tested.

In this study, we characterize genetic variation of a large-bodied mammal that has recently recolonized several Great Basin mountain ranges (Lackey, Beckmann, & Sedinger, 2013). We apply empirical estimates of census size from ongoing wildlife monitoring programmes to inform analyses and use approximate Bayesian computation (ABC) analyses to evaluate three alternative scenarios of demographic history. We then test signals of connectivity using migration rate estimates and genetic bottlenecks to characterize the genetic consequences of recolonization.

1.1 | Study system

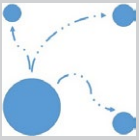
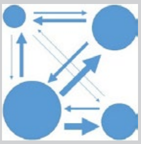
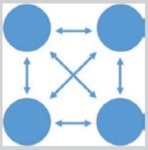
The Great Basin is among the most vulnerable ecosystems in North America (Chambers, 2008). Unfortunately, we still know little about natural patterns of genetic variation and connectivity across this heterogeneous landscape (Riddle, Jezkova, Hornsby, & Matocq, 2014), and many populations may already be imperilled, especially mammals (Beever, Ray, Mote, & Wilkening, 2010). Several species associated with the Great Basin experienced declines during the early and mid-20th century, but some populations of large-bodied mammals have rebounded. For example, by 1940, black bears (*Ursus americanus*) were extirpated from much of their native range but have rebounded and now include >400 individuals (Lackey et al., 2013) that naturally recolonized western Nevada (Figure 1). Considering this well-documented natural rewilding event, this system presents a rare opportunity to assess the genetic consequences of recolonization. In particular, it remains unclear whether recolonized individuals are the product of rare founder events with low connectivity, or alternatively, if recolonized areas maintain high connectivity. Here, we use genetic data to test these alternative scenarios of recolonization and ongoing connectivity. We expect our analyses to yield insight into genetic connectivity among Great Basin montane regions and to provide a comparative system for understanding the genetic consequences of the recolonization process in other species.

2 | METHODS

2.1 | Samples and genetic data

Complete genetic protocols are available in the supplemental materials, but briefly, we generated genotypes for 495 individuals at 23 microsatellite loci. In all reactions, we included positive and negative

TABLE 1 Expected signatures of genetic consequences following alternative modes of recolonization including bottleneck, metapopulation and classical island model. In figures (top), circles represent effective population size, and solid arrows represent scaled migration rates, but broken arrows represent a founder event with no subsequent migration. In the table (below) are expected individual measures under each model

	Genetic consequences models		
	Bottleneck	**Metapopulation**	**Island**
Metric			
Genetic indices (F_{ST}, D)	High low if recent	Low to moderate	Low
Assignment	Excellent poor if recent	Poor	Poor
Bottleneck	Significant	None	None
Effective size (N_E)	Unequal low in recolonized	Unequal	Equal
Gene flow (N_m)	None	Asymmetric	Symmetric

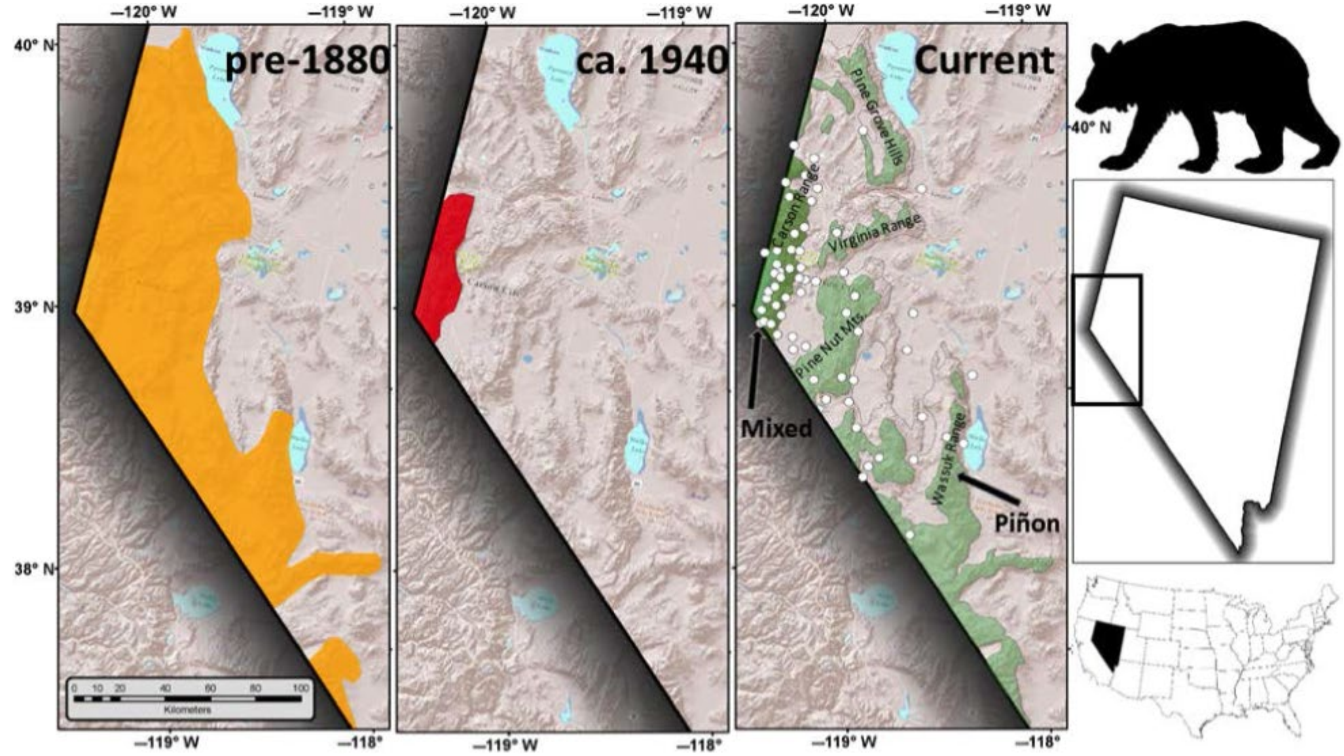


FIGURE 1 Distribution of western Great Basin black bears (*Ursus americanus*) including historical (orange), mid-century (red) and contemporary (white circles). The current distribution is generally associated with montane regions (grey outline) composed of Piñon woodlands (light green) and mixed forests (pine/sprucefir; dark green)

controls with a minimum of two replicate analyses and augmented the nuclear perspective of genetic diversity by generating mitochondrial DNA sequences from a subset of samples.

2.2 | Genetic diversity

We calculated molecular diversity indices for the mtDNA dataset using DnaSP 5.10.01 (Librado & Rozas, 2009) including segregating sites (S), number of haplotypes (N_h), haplotype (h) and nucleotide (π) diversity, and mean nucleotide differences (K). We generated indices of genetic diversity including mean number of alleles per locus (N_A) and mean observed (H_O), and expected (H_E) heterozygosity with GenAlEx 6.5 (Peakall & Smouse, 2012). We tested for linkage equilibrium (LE) and compared H_o to expectations of Hardy–Weinberg equilibrium (HWE) using the chi-squared (χ^2) goodness-of-fit test for the entire set of samples and at a finer scale for each sampled area (Table S2). Considering numerous comparisons ($N = 58$), we adjusted statistical significance using the Dunn–Šidák correction. Finally, we estimated differentiation using F_{ST} and Nei's corrected distance (D).

To assess whether genetic differences are the product of spatial factors, we tested for isolation by distance (IBD) using Mantel tests with the adegenet package for R using Nei's genetic distance (method 1) compared to Euclidean geographic distance (degrees) and assessed significance using a Monte Carlo permutation test (999 replicates; SF 1). When using genetic data, Mantel tests may be inappropriate because results can be influenced by spatial autocorrelation (Guillot & Rousset, 2013). Because we expect spatial autocorrelation in our data, we used correlograms (Diniz-Filho et al., 2013) to aid with interpretation of Mantel tests and gain additional insights into spatial patterns using the ecodist package in R (Goslee, Urban, & Goslee, 2017).

2.3 | Genetic differentiation and population assignment

For most analyses, we assigned individuals to five putative subpopulations based on major geographic areas (Figure 1). To assign individual bears and define subpopulations, we constructed a 1,600-m elevational contour and then assigned individuals within or most proximal to each geographic area. Geographic areas included the (1) Carson Range, (2) Northern Mountains (Peavine Peak, Spanish Springs Peak, PahPah Range), (3) Pine Nut Range, (4) Southern Mountains (Pine Grove Hills, Sweetwater Range and Wassuk Range) and (5) Virginia Range.

We used two complementary approaches to detect regional patterns of geographically based genetic variation including Bayesian clustering (BC) and principal coordinates analysis (PCoA). To assess patterns of genetic structure, we used the program structure v2.3.3 (Pritchard, Wen, & Falusch, 2010) using the standard model that allows for admixture and imposes the F-model (correlated allele frequencies) across populations. We allowed cluster values (K) to vary from 1 to 10, with ten replicate analyses, and a burn-in period of 100,000 with MCMC sampling of 1 million repetitions. We identified optimal number of clusters using two complementary approaches. First, we applied the Δ*K* method but in certain circumstances, such as the presence of hierarchical variation which is expected in this system, the Δ*K* approach may poorly estimate the number of clusters, so we heuristically examined the differences in log-likelihood values among simulations (Pritchard et al., 2010).

The standard structure model uses only genetic information to learn about population structure, but that approach may underperform especially in the case of subtle genetic differentiation likely associated with recent recolonization. To ensure that we reasonably incorporate all prior information and accurately characterize genetic variation, we also used a custom model. For example, we know the sampling locations (geographic areas) and suspect allele frequencies are skewed because of recent recolonizations and presumed reduced gene flow. Therefore, we enforced a population prior, adjusted lambda (λ = allele frequency prior) from 1.0 to 0.1 and modified alpha (α = migration prior) to 0.5.

We used multivariate ordination to characterize the genetic variation among individuals and assess regional patterns of geographic subdivision. We first generated a pairwise genetic distance matrix of individuals using GenAlEx, and then conducted a PCoA by applying the covariance-standardized approach and calculated the mean and standard deviation of dominant axes.

2.4 | Population bottlenecks and N_E

We tested for evidence of recent genetic bottlenecks for each geographic subpopulation using the program Bottleneck 1.2.02 (Piry, Luikart, & Cornuet, 1999). We first assessed whether populations match an infinite alleles model, a strict stepwise mutation model or a two-phase mutation model and applied a range of values for the proportion of multistep mutations (p_g = 0.05, 0.11, and 0.22). We then used a Wilcoxon sign-rank test to assess the presence of excessive heterozygosity and test for characteristic mode shifts in allelic frequencies, both of which often characterize genetic bottlenecks.

Allele-based frequency measures of N_E can be problematic, so we also estimated N_E for each geographic area and the total bear population using the linkage disequilibrium approach in the program NeEstimator 2.01 (Do et al., 2014). For analyses, we removed significantly low frequency alleles (<0.02) derived with a jackknife approach. Our data violate some assumptions of N_E estimators, but Robinson and Moyer (2013) report that linkage disequilibrium approaches maintain performance under relaxed assumptions.

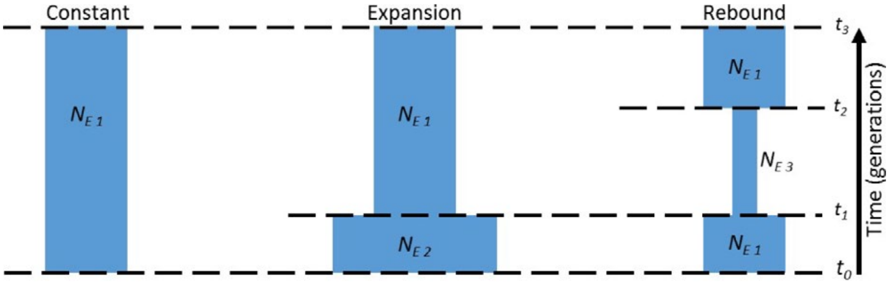
2.5 | Gene flow

We used two approaches to assess gene flow between geographic areas. First, we used F_{ST} measures as an indication of ancestral populations (Epps et al., 2013). While unexpected, some black bears may have gone undetected on isolated mountain ranges and so we used this measure, in combination with mtDNA, as an index of potential undocumented populations. Using $F_{ST,}$ we assume minimal influence from very recent migrations. Simulation studies show this assumption as robust in large-bodied mammals (Epps et al., 2013).Consequently, if we observe high F_{ST} values, we can infer low historical migration rates; however, low F_{ST} values could indicate either sustained migrations (albeit undocumented) or more recent recolonization.

Considering challenges of assessing recent gene flow with F_{ST}, we applied a second approach using a Bayesian estimation of recent migration rates with BIMr 2.0 (Faubet & Gaggiotti, 2008). This approach uses assignment tests to calculate migration rates in the most recent generation by assuming drift-migration equilibrium. BIMr has optimal performance when migration rates are high and when global allele fixation is low (F_{ST} > 0.01), and therefore may offer the best estimate of gene flow even when expected genetic structure is weak. Considering the recent history of recolonization of this system, we expect low F_{ST} and weak population structure and therefore anticipate reasonable performance of this approach. Nevertheless, our data violate some assumptions of this analysis including overlapping generations, samples drawn during a multiyear period and some populations likely failing to conform to drift-migration equilibrium during the previous generation. We treat estimates from BIMr as a relative index of recent gene flow rather than a precise measure from the previous generation (Andreasen, Stewart, Longland, Beckmann, & Forister, 2012; Epps et al., 2013). For analyses, we initiated three MCMC using 1,000,000 iterations, each preceded by a 10% burn-in, a thinning interval of 100, and assessed convergences using trace plots. We calculated the mean, median and 95% confidence intervals of estimates from 10 replicates across all chains.

2.6 | Approximate Bayesian computation

We applied ABC to compare and evaluate alternative demographic scenarios by uniting the mtDNA and microsatellite datasets into a single analysis using DIYABC 2.0 (Cornuet et al., 2014). First, we identified the best-fit model of nucleotide substitution (F81, −lnL = 926.6932) for the mtDNA using the Bayesian information criterion (BIC in jModelTest 2.1.4) (Darriba, Taboada, Doallo, & Posada, 2012). We then model increasingly complex scenarios (Figure 2) ranging from no demographic events and a single N_E (constant), a single event (expansion), to the most complex with two demographic events (decline/rebound). For all scenarios, we set uniform prior distributions (0.3333) and conditions for the sequence of historic events and structure of demographic events ($V_{AR}N_E$) based on historical records and independent census estimates (N_C) from wildlife management programmes (Lackey et al., 2013). Because of a variety of factors including uneven sex ratios, age structure and population fluctuations, N_E is generally smaller than N_C (Allendorf, Luikart, & Aitken, 2013). In bears, N_C/N_E is reportedly between 0.05 and 0.38 (Miller & Waits, 2003; Schwartz, Tallmon, & Luikart, 1998; Tallmon, Bellemain, Swenson, & Taberlet, 2004) and likely fluctuates. Consequently, for a starting point, we applied 20% of N_C estimates and assume a normal distribution with ±3 *SD* (~95%) which is comparable to empirical N_E estimates (see above). For demographic events, we used single uniform priors' with ±5 generations. Other priors were left default. For all scenarios, we assume random mating, overlapping generations and apply the generalized stepwise



10.2 (09.18–11.31) **10.6** (09.49–11.67) **79.2** (77.57–80.78)

	Priors		
	Mean	SD	95% Distribution
$N_{E\,1}$	52	6	34-71 
$N_{E\,2}$	105	12	68-142 
$N_{E\,3}$	15	5	1-30 
t_0	0	0	N/A
t_1			10-20 
t_2			35-45 
t_3			55-65 

FIGURE 2 Alternative scenarios of demographic history for black bears (*Ursus americanus*) in the western Great Basin assessed using approximate Bayesian computation including (1) constant population, (2) population that recently expanded or (3) population that underwent a mid-century decline and recent rebound. Estimates of posterior probabilities are below each scenario with 90% highest posterior density intervals (logistic approach, N = 10k) in parentheses generated from 1 M simulations. Within each scenario, prior density parameters were derived from empirical data (N_C = 262, SD = 31, Lackey et al., 2013) including effective population sizes (N_E = width) and duration of events (height—uniform prior). Dashed lines represent timing (t_x—generational units) of hypothesized changes in demography (effective population size—N_E). Specific parameters are presented in the table below where NE distributions were sampled using ±3 normal standard deviations (SD), but t_x was sampled from a single uniform prior

mutation model. We collected four-one-sample summary statistics (Cornuet et al., 2014) and simulated 1 million datasets for each scenario drawing from the specified prior distributions (Figure 2), and estimated the posterior probabilities by recovering the closest 1% of simulated data. From this sample, we calculated four within, and six among, population summary statistics (SS) using logistic regression (following logit transformation). We performed model checking using principal components analysis of the population SS and identified the observed value among the closest values of simulated data. Finally, we evaluated confidence in model choice using linear discriminant analysis of pseudo-observed datasets (pod) derived from 999 replicates estimating prior and posterior predictive error rates (Cornuet et al., 2014).

3 | RESULTS

3.1 | Genetic diversity

The microsatellite dataset included 449 samples that consistently amplified for 19 polymorphic loci, none of which failed multiple goodness-of-fit tests for deviation from HWE (Table S2). Across loci, we observed 120 alleles (Table 2) with a mean of 4.495 alleles per locus (SD = 2.768, min = 2, max = 12), 85.40 alleles per subpopulation (SD = 18.474, min = 61, max = 112), plus private alleles for 4/5 subpopulations (except Virginia Range). Finally, we documented equivocal observed and expected heterozygosities for all loci, allelic richness of 56 (Table 2) and low pairwise genetic differentiation ($F \le 0.05$, $D \le 0.025$, Table S3).

The mtDNA dataset (Table S4) contained two haplotypes, the product of a single variable site (parsimony informative) and an alignment gap resulting in low nucleotide diversity (π = 0.00077). Both haplotypes were shared among all area (Fig. S2).

Mantel test revealed a significant (F = 1,586, $p \ll .001$, df = 100,574) positively autocorrelated pattern (y = 6.1990 + 0.3127, Fig. S1), but that describes little of the observed genetic variation (RSE = 0.7486, R^2 = 0.01552). Nevertheless, dense sampling and permutation tests permit us to reject the null hypothesis that genetic distance and geographic distances are unrelated ($p < .001$, $r = .1246$), except among neighbouring bears (SF1).

3.2 | Genetic differentiation and population assignment

We documented a K = 1 as the optimal characterization of genetic variation using the standard structure model (Fig. S3). However, we witness improved performance using a custom model, but assignment probability of individuals to subpopulations was low (~30%).

Multivariate ordination of nuDNA revealed two vectors sufficient to explain 12.23% of observed genetic variation, but no strong clustering of individuals (Figure 3b). Furthermore, the standard deviation of scores showed large overlap among the geographic areas, largely consistent with the model-based approach and measures

TABLE 2 Population summaries of genetic variation from 19 microsatellite markers sampled from five geographic subpopulations of black bears (*Ursus americanus*) from the western Great Basin. Indices include number of sampled individuals (*N*), total number of alleles (TN_a), observed private alleles (P_a), average alleles (N_a), allelic richness ($R_{(g)}$—rarified measure), observed (H_o) and expected (H_e) heterozygosity, and percentage of polymorphic loci (%P)

Carson	294	112	17	5.895	56.46	0.479	0.506	100
Pine Nut	87	91	4	4.789	56.13	0.459	0.502	100
Virginia	7	61	0	3.211	55.33	0.466	0.448	89.5

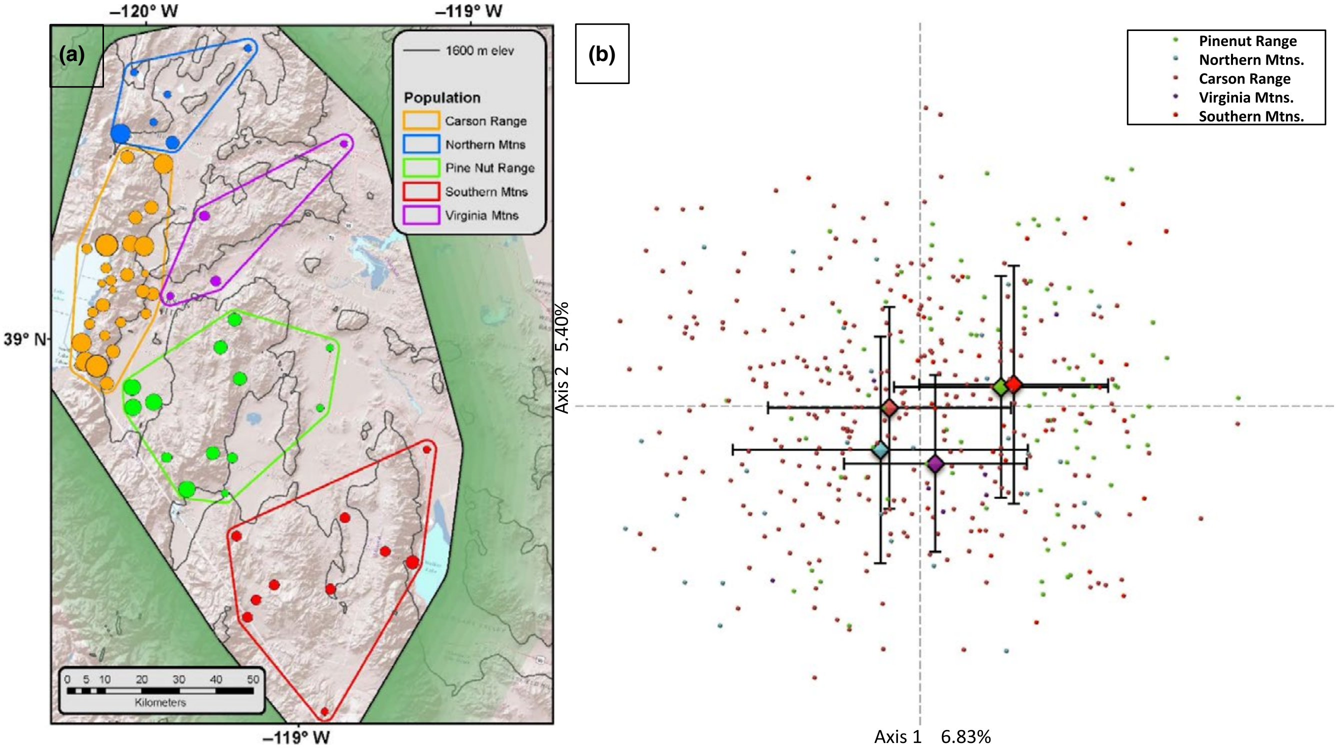


FIGURE 3 (a) Black bear (*Ursus americanus*) sampling locations and sample sizes used for characterizing genetic variation in five geographic subpopulations (colours) in the western Great Basin. The 1,600-m elevation isocline was used for delineating geographic subpopulations. (b) Principal coordinates analysis (PCoA—using covariance and standardization) of genetic variation of western Nevada black bears. Small circles represent sampled individuals ($N = 449$); large diamonds are the mean and standard deviation for each geographic subpopulation. When combined, the primary axes account for 12.23% of the observed genetic variation

of genetic differentiation (Table S3). More subtle genetic patterns indicate a general north-to-south orientation of genetic variation (Figure 3b).

3.3 | Population bottlenecks and N_E

Analyses of genetic bottlenecks revealed that observed allelic frequencies failed to deviate from equilibrium (Figure 4), and all subpopulations had observed (0.400–0.895) Wilcoxon test probabilities greater than expected (0.05), so we reject the hypothesis of recent genetic bottlenecks (Luikart et al., 1998). Estimates of N_E revealed that the source area (Carson Range) had the largest effective population size (38), and all recently recolonized areas were significantly lower, each with values <10, and with the Virginia Range lowest (Figure 5). The estimate of N_E for all black bear in western Nevada is 41 individuals ($N_C/N_E = 0.156$).

3.4 | Gene flow

Estimates of gene flow revealed asymmetric rates among areas occupied by black bear in western Nevada (Figure 5). Net rates of emigration/immigration were similar between the Pine Nut Range, Northern Mountains and Southern Mountains, but the Virginia Range had low emigration (≪0.001) with high immigration. In

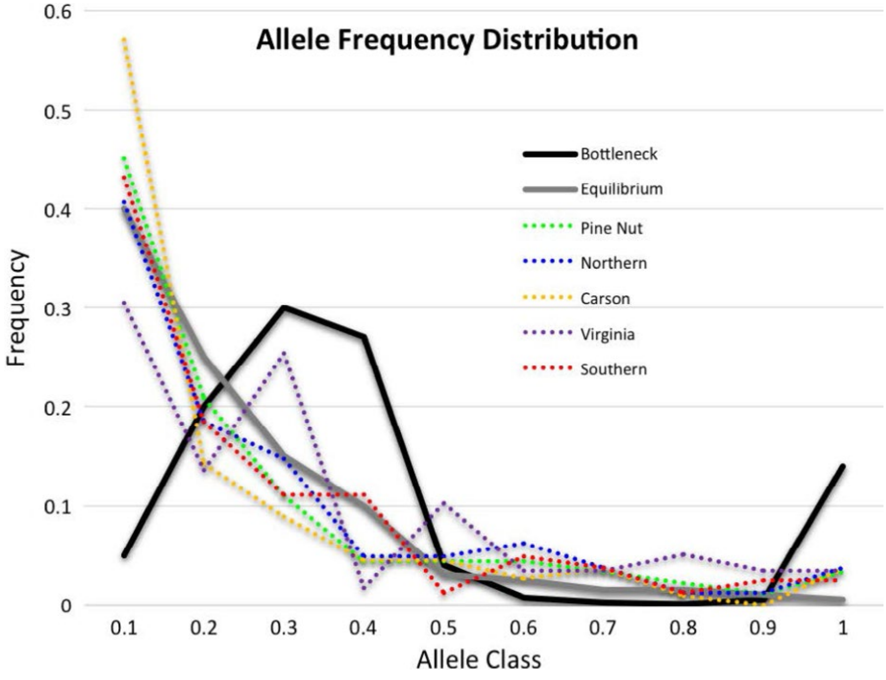


FIGURE 4 Expected (solid) and observed (dotted) allele frequency distributions for black bears (*Ursus americanus*) in the western Great Basin. Expected distributions were generated using computer simulations under the assumption of mutation-drift equilibrium (L-shaped distribution—grey) or a recent bottleneck (allele frequency distortion—black). All subpopulations fail to deviate from equilibrium (WTP—Wilcoxon test probability; *p* = .05)

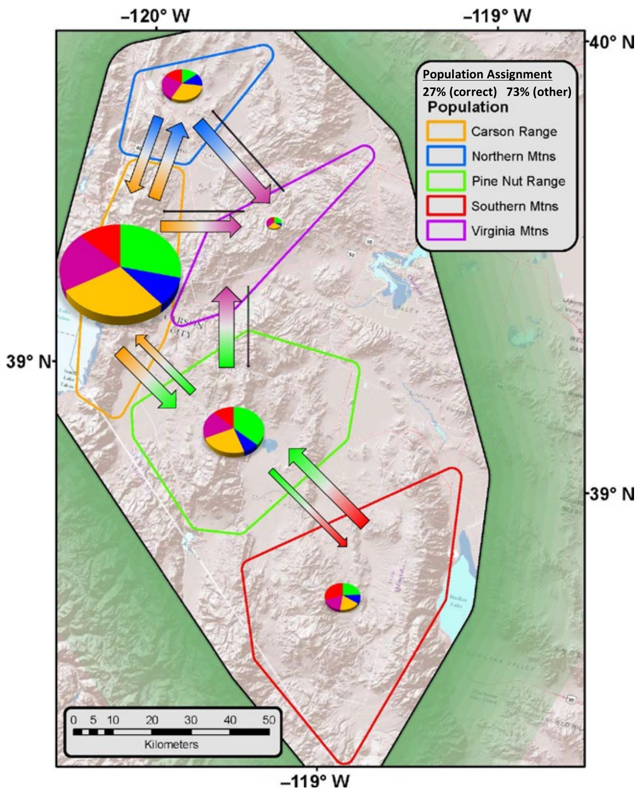


FIGURE 5 Population assignment, effective population size (N_E) and relative gene flow (arrows) among adjacent subpopulations (see appendix for full matrix) of Nevada black bears (*Ursus americanus*). Pie chart sizes are scaled by N_E, with individual slices representing population assignment. Arrows represent relative rates of gene flow scaled by recipient NE, and colour coded by source and recipient populations

contrast, the Carson Range had high net emigration rates with low net immigration. Most pairwise comparisons had suitable confidence intervals for estimating migration rates with the exception being all pairwise comparisons with the Virginia Range, likely due to small sample sizes.

3.5 | ABC simulations

In general, ABC simulations performed well for comparing alternative demographic scenarios which identified the Rebound Model as having the highest posterior density probability (Figures 2 and S4). This model also had high confidence in model choice considering the posterior predictive error was low (S1 = 0.02, S2 = 0.08, S3 = 0.90) compared to the prior predictive error (S1 = 0.31, S2 = 0.34. S3 = 0.35) derived from 999 pseudo-observed datasets (pods).

4 | DISCUSSION

Many populations of North American wildlife, especially large mammals, have experienced dramatic declines due to overharvest, disease and land use change. Some wildlife populations have proven resilient and are now rebounding largely because of improved conservation and management programmes. Black bears of the Great Basin are a system that appears to be in a phase of rebound, including natural recolonization of portions of their once-extirpated range. Assessing the genetic consequences of recolonization is central to developing conservation strategies that will support the unique demographic and connectivity dynamics of these rebounding populations.

4.1 | Genetic variation, population structure and effective population size

We detect weak differentiation among black bears in western Nevada. For example, we fail to reject the hypothesis of *K* = 1 (Fig. S3) when we use the standard structure model. While performance improves with a custom model (Fig. S3), we still observe poor assignment at $K = 5$ (Fig. S3). This approach to BC is designed to improve discrimination among populations even when the signal of population structure is weak (Hubisz, Falush, Stephens, & Pritchard, 2009; Pritchard et al., 2010), and it has been useful for studying population structure in other large mammals with low genetic differentiation (Pease et al., 2009), and in systems with uneven sampling (Puechmaille, 2016). However, this approach can be prone to bias because of high parameterization and so should be treated with caution (Latch, Dharmarajan, Glaubitz, & Rhodes, 2006; Pritchard et al., 2010). In this study, we conclude that there is weak structure in this system with few genetic differences useful for distinguishing subpopulations of black bears. Furthermore, we detected low indices of differentiation (i.e., Nei's distance and F_{ST}, Table S3) and geographic overlap of mtDNA haplotypes (Fig. S2). Distance-based multivariate ordination shows genetic similarity (Figure 3B) among all subpopulations. Despite the lack of genetic differentiation among isolated mountain ranges, there is a subtle spatial trend in the PCoA from north to (Northern and Carson) south (Southern and Pine Nut), and this pattern is likely the by-product of IBD (Fig. S1) where geographically adjacent localities are genetically most similar.

Often, wild populations show a significant pattern of IBD that accounts for observed genetic variation, and we detect a significant correlation between genetic and geographic distance matrices (Fig. S1). However, Euclidian distance accounted for a small portion of genetic variation ($R^2 < 0.02$) and so is insufficient to make meaningful comparisons. Additional tests are needed to determine whether other factors (e.g., slope, land cover) are important in predicting patterns of black bear genetic variation as is often the case in other populations of bears (Cushman, McKelvey, Hayden, & Schwartz, 2006; Murphy, Evans, Cushman, & Storfer, 2008).

In this study, we also find no clear genetic signature of remnant or undocumented populations of black bears from samples obtained in recolonized areas. If remnant, albeit undocumented, individuals persisted in the western Great Basin through the last century, we may expect mtDNA haplotypes that differ from haplotypes observed in the Sierra Nevada. However, we detected shared mtDNA haplotypes among all areas (Fig. S2). Likewise, remnant bears in the Great Basin would be expected to harbour distinct nuDNA alleles that would contribute to genetic differentiation between source and recolonized areas (Sierran versus Great Basin), but we detect no such subdivision. We do detect a limited number of private nuclear alleles in the recolonized range; however, the origin of these private alleles remains unclear, but we suspect they are the product of sampling effects. For example, private alleles in the recolonized range may be (1) rare alleles that we failed to sample in the source area or that (2) we failed to sample all possible source regions, that is, the Carson Range may not be the only Sierran region to which Great Basin bears are, or recently have been, genetically connected. Finally, (3) these alleles could be the product of new mutations, although considering the presumed mutation rates of these loci (Kristensen, Faries, White, & Eggert, 2011; Meredith, Rodzen, Banks, & Jones, 2009; Puckett et al., 2014) we consider this latter hypothesis unlikely. Regardless, the overall lack of evidence of genetic distinction of subpopulations strongly suggests that historical populations were indeed extirpated during the mid-20th century from the western Great Basin.

Because we detected no remnant signature of historical populations across the western Great Basin, coupled with a weak pattern of genetic structure among isolated mountain ranges, we conclude that black bears have experienced recolonization. When combined, these patterns suggest that black bears have recently dispersed across inhospitable environments. For example, the Carson Range and the northern subpopulation are separated by a major interstate highway (I-80), medium-sized river (Truckee River) and an urban environment (Reno-Sparks metropolitan area; Figures 1 and 4a). Nevertheless, these subpopulations maintain low pairwise F_{ST} (0.005) and low corrected genetic distance (0.001). This pattern is similar for other comparisons in this system (Table S3) despite at least one major obstacle to dispersal in all pairs. Consequently, while barriers such as highways often reduce gene flow in other large-bodied mammals (Epps et al., 2005), black bears in the western Great Basin appear to occasionally traverse these obstacles. Even in the presence of barriers, black bears often exhibit low genetic differentiation. For example, Central Interior Highlands black bears also show low genetic differentiation ($F_{ST} = 0.01–0.07$; (Puckett et al., 2014), and in northern Idaho, two populations are separated by unfavourable habitat (Murphy et al., 2008), but maintain low genetic differentiation ($F_{ST} = 0.02–0.06$; (Cushman et al., 2006). Therefore, it appears that spatially proximate populations may frequently maintain low differentiation, even when separated by inhospitable environments. While additional tests are needed, western Nevada black bears do not appear to have genetic differences that can be attributed to barriers, and the low differentiation is likely the product of recent recolonization and ongoing connectivity.

4.2 | Demographic history

Bayesian analyses of genetic variation are burgeoning in part because of the ability to compare alternative scenarios and the ability to integrate independent datasets into genetic analyses (Beaumont, 2010; Cornuet et al., 2014; Luikart, Ryman, Tallmon, Schwartz, & Allendorf, 2010). One particularly rich source of independent data comes from population monitoring programmes by natural resource agencies. In this study, we detect that genetic patterns match expectations from >100 years of census data and that contemporary N_C/N_E in western Nevada black bears as 0.156 (empirical estimate $N_E = 41$ and N_C of 262 from Lackey et al. (2013)). N_C/N_E in bears ranges from 0.05 to 0.38 (Miller & Waits, 2003; Schwartz et al., 1998; Tallmon et al., 2004) but likely fluctuates (Luikart et al., 2010). It remains unclear if the N_C/N_E ratio fluctuates in this system, but posterior distributions of N_E failed to differ significantly from prior distributions (Supplementary Materials), suggesting our estimates were unbiased and that the ratio remained unchanged during modelling.

The signature of demographic change is important because it indicates that bears retain the genetic signature of a historic decline followed by a demographic rebound. While N_C/N_E is low compared to some other bear populations (Miller & Waits, 2003; Schwartz et al., 1998; Tallmon et al., 2004), this signature may mean that reproductive success among individuals varies resulting in fewer effective individuals (see more below). While our assessments of genetic signatures detected demographic decline and rebound, subpopulations may be at risk of extirpation because of too few effective individuals, and so assessments of connectivity are required.

4.3 | Genetic connectivity

We document a signal of asymmetric gene flow in this system. Specifically, bears from the Carson Range have the greatest emigration (cumulative scaled rate = 0.422), although the Virginia Range has the lowest (cumulative rate <0.001); other subpopulations had similar rates (Figure 5, cumulative rate 0.103–0.277). The Virginia Range also has the highest immigration with a cumulative rate of 0.83, but this measure has wide confidence intervals, likely because of too few samples (N = 7). In contrast, the Carson Range has low immigration (<0.01, Figure 5). Considering the Carson Range (mixed-conifer habitats) is the likely ultimate source for recolonizations, we interpret high emigration and low immigration as a signal of source–sink dynamics. However, the signal of similar emigration/immigration rates between other areas (i.e., Pine Nut and Southern) indicates migrants move more frequently among piñon-juniper woodland habitats despite interspersed unfavourable habitats. Consequently, these contrasting patterns of estimated migration rates suggest asymmetric connectivity where the Carson Range subpopulation serves as the dominant source of migrants to other areas. However, once colonized, individuals appear to move largely unimpeded between piñon-juniper woodlands with few individuals migrating back to the source areas composed of mixed-conifer forests (Carson Range). Considering the biology and behaviour of bears, including their recent history, this pattern is not unexpected in this system, but differs from other large-bodied carnivores in the Great Basin. For example, Andreasen et al. (2012) found that mountain lion (*Puma concolor*) also have asymmetric gene flow, but migrants appear to migrate towards mixed-conifer forests from piñon-juniper woodlands, but this pattern may be partially attributable to anthropogenic factors including excessive persecution and ongoing predator control programmes.

We tested alternative models of genetic connectivity using estimates of gene flow and genetic bottlenecks combined with characterizations of genetic variation among individual subpopulations. Taken together, results of these assessments indicate that black bears in the western Great Basin best match a model of a metapopulation (Table 1). When we convert migration rates using N_E, we detect >50 effective individuals that have emigrated out of the mixed-conifer forests (Carson Range) to the piñon-juniper woodlands, with only three effective individuals returning. This pattern has important implications for connectivity, especially considering that anthropogenic factors likely contribute to how bears navigate during dispersal. For example, bears associated with urbanized areas have access to anthropogenic food resources (e.g., garbage) resulting in smaller home ranges and higher survival (Beckmann, 2002), different selective foraging behaviours (Hopkins, Koch, Ferguson, & Kalinowski, 2014), higher age-specific fecundity (Beckmann & Lackey, 2008) and altered activity patterns (Beckmann & Berger, 2003). Consequently, urban-associated bears may be affected less by anthropogenic barriers than wildland bears.

In this study, genetic data have permitted the direct measurement of connectivity among black bears in western Nevada, thereby allowing inferences of dispersal between occupied areas. If connectivity of bears is allowed to remain intact, then we can expect continued, even growing, dispersal among occupied areas. What remains unclear, however, is following recolonization, whether demographic growth rates (new individuals in subpopulations) are primarily the product of local recruitment (survival and reproduction of residents) or connectivity (dispersal among subpopulations).

Genetic data provide little direct evidence of local recruitment without characterizing the genetic variation of all reproductive individuals within subpopulations (parentage analyses). Sampling all reproductive individuals can be difficult, but uniting genetic data with capture–mark–recapture approaches may help decouple these complex signatures (Allendorf et al., 2013). Regardless, future management will require careful characterization of the demography that includes both local recruitment and connectivity to ensure that both contribute to persistence.

5 | CONCLUSIONS AND IMPLICATIONS

This study represents the first characterization of genetic variation of black bears in Nevada, the first model-based characterization of connectivity in bears and one of the few empirical examples of genetic consequences of natural recolonization in large-bodied mammals. Based on our results, black bears in the western Great Basin appear to maintain levels of connectivity sufficient to preclude genetic bottlenecks following recolonization. Furthermore, we conclude that black bear in the western Great Basin best represents a genetic metapopulation. These results are both timely and relevant because of projected climate change and because this region is expected to have rapid anthropogenic growth within the next 20 years. Specifically, the effects of the Great Basin environment warming and drying (Chambers, 2008) combined with increased urbanization and land use changes will likely alter the connectivity of many species in this region, including black bears. Therefore, special steps to maintain connectivity are required now, before additional impacts are realized. For example, wildlife-crossing structures are useful for providing genetic connectivity of many species including black bears (Sawaya, Kalinowski, & Clevenger, 2014). Furthermore, additional studies that build on these results should be a priority. First, a more detailed analysis of the link between landscape variables and genetic variation (Atwood et al., 2011; Cushman et al., 2006) to identify how habitat changes, including habitat fragmentation, impacts these recolonized populations. Second, a more complete understanding of how the degradation of migration corridors (reduced gene flow), or the presence of artificial forests (enhance gene flow), influences connectivity would be valuable. If bears continue to naturally recolonize their previously extirpated range, thereby rewilding the Great Basin, maintaining the potential for connectivity must be a management priority in this system and across the region.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

This is to be finalized once manuscript accepted for publication.

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BIOSKETCH

Our team has a research focus on the ecological variation and evolutionary genetics of western North American vertebrates and is interested in uniting field data with genetic tests to provide novel perspectives for conservation and management.

Author contributions: C.W.L. & J.P.B. collected samples; J.L.M. conducted laboratory work; and all authors contributed to conceptualizing the research ideas and writing of the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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