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LETTER

Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep

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Abstract

The rapid expansion of road networks has reduced connectivity among populations of flora and fauna. The resulting isolation is assumed to increase population extinction rates, in part because of the loss of genetic diversity. However, there are few cases where loss of genetic diversity has been linked directly to roads or other barriers. We analysed the effects of such barriers on connectivity and genetic diversity of 27 populations of *Ovis canadensis nelsoni* (desert bighorn sheep). We used partial Mantel tests, multiple linear regression and coalescent simulations to infer changes in gene flow and diversity of nuclear and mitochondrial DNA markers. Our findings link a rapid reduction in genetic diversity (up to 15%) to as few as 40 years of anthropogenic isolation. Interstate highways, canals and developed areas, where present, have apparently eliminated gene flow. These results suggest that anthropogenic barriers constitute a severe threat to the persistence of naturally fragmented populations.

Keywords

Gene flow, genetic diversity, habitat fragmentation, metapopulation, *Ovis canadensis*, road.

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INTRODUCTION

As the level of connectivity among human populations continues to increase, natural populations of plants and animals are becoming increasingly isolated. Today the earth's surface is partitioned by an estimated *c.* 28 million km of highways (CIA 2003) that restrict the movement of many species (Trombulak & Frissell 2000; Underhill & Angold 2000). This loss of connectivity is suspected to impede exchange of individuals among populations, thereby accelerating the loss of genetic diversity because of genetic drift (Frankel & Soule 1981; Hedrick 2005). Reduced genetic diversity is likely to increase population extinction rates both in the short term (because of inbreeding, Saccheri *et al.* 1998; Westemeier *et al.* 1998; Coltman *et al.* 1999) and in the long term by reducing evolutionary potential, i.e. the ability of a population to adapt to future changes in biotic and abiotic factors such as climate change (Frankel & Soule 1981; Lande 1998; Fraser & Bernatchez 2001; Hedrick 2005). However, recently constructed barriers have rarely been found to affect genetic diversity in natural populations, particularly for long-lived, large-bodied species (e.g. Kyle & Strobeck 2003; Sumner *et al.* 2004). While roads have been shown to restrict gene flow for species with small body size or relatively low vagility such as amphibians (Reh & Seitz 1990) and beetles (Keller & Largiader 2003), there is growing concern that a much wider variety of taxa may be affected (e.g. Kramer-Schadt *et al.* 2004; Malo *et al.* 2004).

The objective of this study was to assess the effects of major highways and other recently constructed anthropogenic barriers upon genetic diversity in a metapopulation of *Ovis canadensis nelsoni* (desert bighorn sheep). In the desert regions of California, local populations of this long-lived, vagile mammal are often less than 50 individuals (Torres *et al.* 1994). Restricted largely to the steep, rocky mountain ranges that are scattered across the region, these populations are demographically independent and naturally fragmented by the intervening desert (Bleich *et al.* 1990). As resources are variable and local population extinctions common (Epps *et al.* 2004), some connectivity among populations is presumed essential to maintain the regional bighorn sheep metapopulation (Bleich *et al.* 1996). However, the southwest USA has been subject to an increasing degree of urbanization by humans, marked by widespread construction

of interstate highways and water canals in this desert region over the last 40–70 years. Anecdotal evidence suggests that bighorn sheep rarely cross these continuously fenced barriers (Bleich *et al.* 1996). Thus it is likely that these barriers on the landscape have reduced connectivity among populations of desert bighorn sheep and possibly many other terrestrial species.

We examined putatively neutral genetic variation across desert bighorn sheep populations in southeastern California (Fig. 1) to assess whether human-made barriers have affected dispersal and genetic diversity to a significant degree. We also defined the geographical scale of current gene flow among these populations and considered the conservation implications of continuing anthropogenic fragmentation.

METHODS

The study area was comprised of the central Mojave, southern Mojave and Sonoran Desert regions of California. Habitat quality for desert bighorn sheep in these arid areas was strongly affected by the spatial and temporal variation in climate and population turnover is high (Epps *et al.* 2004). Apparent dispersal barriers erected in the 20th century include the Colorado River Aqueduct (constructed in the 1930s), urban development, the establishment of large mining operations in Lucerne Valley, the portion of State Highway 62 with four lanes and a concrete median barrier, and interstates 10, 15 and 40 (constructed in the 1960s) (Nystrom 2003). These barriers are largely continuous and have direct physical impediments to locomotion by bighorn sheep, including fences and steep concrete walls. Underground portions of the Colorado River Aqueduct (passing beneath several populations in the southeastern part of the study area) were not considered to be barriers. Major highways were by far the most common barriers between study populations.

We collected genetic samples across the study area during 2000–2003 from 27 populations with varying levels of anthropogenic isolation (Fig. 1). Estimated median population size for these populations was 38 individuals, range was 12–300 (Torres *et al.* 1994). Populations were defined as previously in a geographical information system (GIS) (Torres *et al.* 1994; Epps *et al.* 2004, 2005a), based upon the topographical features of the mountain ranges where they are found. We collected samples from all known populations within the focal study area, except five ranges containing individuals translocated from other populations in the region (Torres *et al.* 1994) (Fig. 1).

We used faecal pellets as the primary source of genetic material, obtained mostly during summer months when desert bighorn sheep congregate at water sources. We collected fresh pellets from observed bighorn sheep or selected the most recent-appearing pellets in the vicinity. Faecal samples were air-dried and stored in paper bags in a dry environment. We also obtained blood and tissue samples from bighorn sheep captured by the California Department of Fish and Game or killed by hunters during 2000–2004. We extracted genomic DNA from faecal samples using a modified DNA Stool Mini-Kit™ (Qiagen, Valencia, CA, USA) protocol (Wehausen *et al.* 2004), and from blood and tissue samples using DNEasy Tissue Kits™

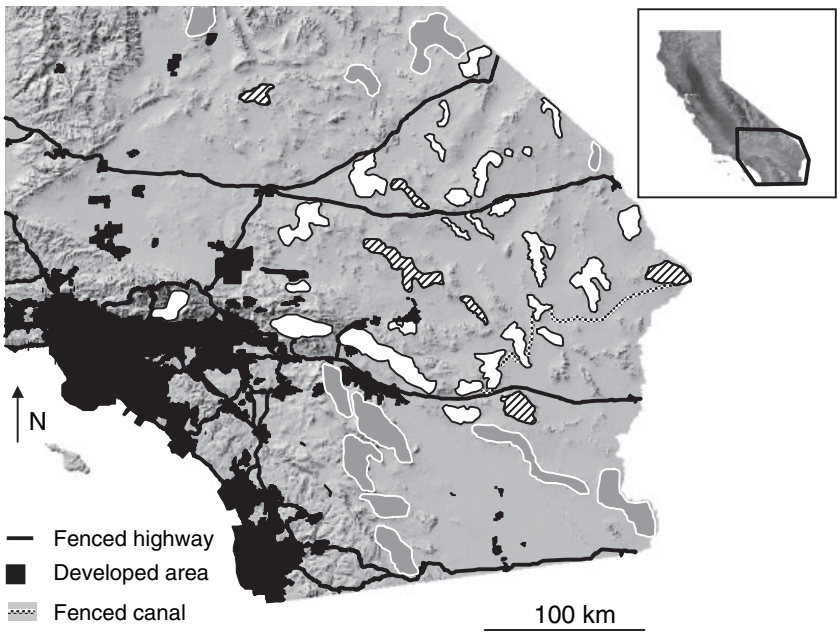

Figure 1 Topographical map of southern California with location and approximate size of the 27 desert bighorn sheep populations sampled (white polygons). Barriers, including canals, interstate highways, freeways, and urban areas, are represented in black or checkered (above-ground portions of the Colorado Aqueduct) patterns. Artificially translocated populations (cross-hatched) and other extant populations where sampling did not occur are also depicted (light grey polygons). Barriers outside the area of sampled populations are not fully represented.

(Qiagen). Before genotyping, we assessed extraction quality by amplifying a *c.* 200-bp fragment of nuclear DNA from the zinc-finger protein gene [Appendix S1(a)]. We visualized the amplification product on 2% agarose gels pre-stained with ethidium bromide; samples generating weak amplifications were not used in further analyses.

We genotyped 14 dinucleotide microsatellite loci for each DNA extraction [Appendix S1(b)]. We conducted a minimum of four replicate polymerase chain reactions (PCRs, Mullis *et al.* 1986) per faecal sample per locus to minimize genotyping errors resulting from degraded DNA (Taberlet *et al.* 1999), and conducted two replicate PCRs for blood and tissue samples. Alleles included in the final consensus genotypes were observed at least twice; if observed only once, an additional four replicates were conducted. We included two negative controls and two positive controls (samples with known genotypes) with every 96 PCR reactions as checks for contamination and to standardize genotypes among experiments.

We estimated the probability of an erroneous genotype because of allelic dropout (selective amplification of only one allele in a heterozygote because of low amounts of template DNA, Taberlet *et al.* 1999). We accomplished this by summing the observed number of allelic dropouts for each locus, and dividing this sum by the number of successful PCR reactions (i.e. the presence of an amplification product) for heterozygous individuals (allelic dropout could only be identified in the case of individuals determined to be heterozygous). Because we had a minimum criterion that each allele per sample per locus had to be observed at least twice (at least two successful replicate PCR reactions with identical results), we squared each per-locus dropout rate to estimate the probability of two dropouts in the same sample. We then summed these squared dropout rates over all loci, and added the average probability of a false allele over 14 loci (calculated from observed rates) to obtain our final estimated probability of a genotypic error per individual. While this method does not account for variability among samples (e.g. Miller *et al.* 2002), we assumed that pre-screening of extractions limited sample variability to a large degree.

We limited further data analyses to samples for which complete genotypes were obtained at all loci. We used the probability of identity (P_{ID}) to identify and eliminate duplicate genotypes resulting from the collection of more than one faecal sample from some individuals. DNA extractions from different faecal samples were inferred as originating from the same individual if the combined P_{ID} for a full-sib relationship was estimated at $< 10^{-2}$ using GIMLET (Valiere 2002), at the number of loci matching between a pair of different DNA extractions (which could be any number of loci less than the maximum of 14 employed in this study). This threshold level of P_{ID} was chosen because most population sizes were estimated at < 100 individuals (Torres *et al.* 1994); 10^{-3} was used for populations > 100. This analysis was undertaken in two steps; first within each population, and then subsequently for all populations combined and treated as a single panmictic population (after removal of all but one of each unique genotype in each population), to detect if any individuals were sampled in more than one population. We assessed the final data set obtained in this manner for any significant deviations from linkage disequilibrium and the expected Hardy–Weinberg genotype frequencies in each population using GENEPOP (Raymond & Rousset 1995).

We also assessed the diversity of mitochondrial DNA haplotypes in each population. Female bighorn sheep are less likely to move between mountain ranges (Festa-Bianchet 1991; Jorgenson *et al.* 1997); therefore maternally transmitted mitochondrial DNA provided an opportunity to assess female dispersal patterns. After identifying unique samples using the microsatellite data, we sequenced 515 nucleotides in the mitochondrial control region from each individual (except three samples that failed to amplify) [primers and protocols are described in Appendix S1(c)]. We sequenced all samples in both forward and reverse directions, editing and aligning them manually, to minimize sequence ambiguities. We used the number of unique haplotypes present in each population as a measure of female-mediated genetic diversity. To correct for variation in sample size, we subsampled each population 100 times using the minimum sample size and calculated the average number of unique haplotypes detected per population.

From the microsatellite data, we estimated the degree of genetic divergence among populations as F_{ST} (and thus Nm) for each population pair using GENEPOP (Raymond & Rousset 1995). F_{ST} rather than R_{ST} (Slatkin 1995) was used because F_{ST} is a more appropriate statistic for 'stepping stone' population models and systems where migration rate exceeds mutation rate (Hardy *et al.* 2003), as is most likely for these desert bighorn sheep populations given numerous observations of colonizations and dispersal between mountain ranges (e.g. Epps *et al.* 2005a,b). Furthermore, F_{ST} performs better when number of loci < 20 (Gaggiotti *et al.* 1999). We used allelic richness (the average number of alleles per locus or A) as our measure of genetic diversity in each population. We used FSTAT (Goudet 1995) to correct A for differences in sample size, as recommended by Leberg (2002). The smallest population sample size was employed as the global sample size.

To determine if human-made barriers (see below) had affected population genetic diversity, we used information theoretic model selection techniques (Burnham & Anderson 1998) to test multiple regression models incorporating either of two estimates of the degree of isolation for each population. We estimated isolation as (i) the harmonic mean

of the geographical distance to the nearest three populations (e.g. Harrison & Ray 2002), which weights the mean towards the smallest distance, or (ii) the harmonic mean of the geographical distance to the nearest three populations, but with a 'barrier effect distance' added to the geographical distance between each population pair separated by a human-made barrier. These measures are referred as $\text{isolation}_{\text{distance}}$ and $\text{isolation}_{\text{distance+barriers}}$.

To quantify the above-mentioned barrier effect distance, we estimated the reduction in the relative gene flow parameter (Nm) caused by barriers among our study populations. The barrier effect distance was defined as the geographical distance yielding an equivalent decrease in the estimate of Nm. We first defined barriers as fenced highways, canals and areas of high-density urban development, and added them to the above employed GIS map. We then employed multiple regressions on all pairwise population comparisons to estimate the degree of correlation between geographical distance and Nm among populations that were (i) separated by human-made barriers and (ii) those that were not. Populations were considered as separated by human-made barriers if a straight line between the two closest edges of the population polygons intersected such a barrier. Connecting lines for all pairwise comparisons were generated in the GIS (Jenness 2004) and overlaid on the barrier map to determine which lines intersected barriers. Interpopulation geographical distances were estimated as the shortest distance between the edges of each population polygon (Jenness 2004).

Nm was estimated as $[F_{ST} = 1/(1 + 4\ Nm)]$ (Wright 1921). The difference between the intercepts of the y-axis in the two regressions (denoted as ΔNm) was inferred to result from the effect of human barriers on the degree of genetic isolation (Fig. 2). Finally, we used the coefficient of the regression of population pairs without barriers ($\text{slope}_{\text{no barriers}}$) to estimate the barrier effect distance (in km) as $\log(\text{barrier effect distance}) = \Delta Nm/\text{slope}_{\text{no barriers}}$.

After defining these two measures of population isolation ($\text{isolation}_{\text{distance}}$ and $\text{isolation}_{\text{distance+barriers}}$), we tested which measure explained the most variance in both A and mtDNA haplotype diversity. For both sets of genetic data, we used Akaike's Information Criterion with the small sample size correction (AIC_c) and Akaike weights (Burnham & Anderson 1998) to infer the best regression models. We estimated the overdispersion correction factor ($\hat{c}$) from the deviance of the most saturated model, as described by Lindsey (1999), to ensure that AIC_c rather than the quasi-likelihood information criterion (QAIC_c) was most appropriate. We also tested whether other factors such as population polygon area and estimated current population size (which affects the rate of genetic drift) improved regression models.

We estimated the rate of reduction in genetic diversity (A) in those populations affected by human-made barriers by comparing the difference in the predicted level of genetic diversity with the existent barriers (obtained from the regression of A on $\text{isolation}_{\text{distance+barriers}}$ described above), and the predicted level of genetic diversity using the same equation but removing the barrier effect for each population. The resulting difference was then extrapolated over the average estimated age of the barriers.

We also analysed pairwise estimates of Nm using partial Mantel tests (Smouse *et al.* 1986; Manly 1991) to determine whether relative gene flow was affected by barriers, and at what spatial scale. We repeated this analysis using F_{ST} for comparison, although F_{ST} appeared to be subject to very high overdispersion in other analyses of this data set (not shown). Nm represents the amount of gene flow in an idealized Wright–Fisher island model that would yield the observed degree of genetic heterogeneity. Hence, Nm cannot be inferred to represent an estimate of the actual

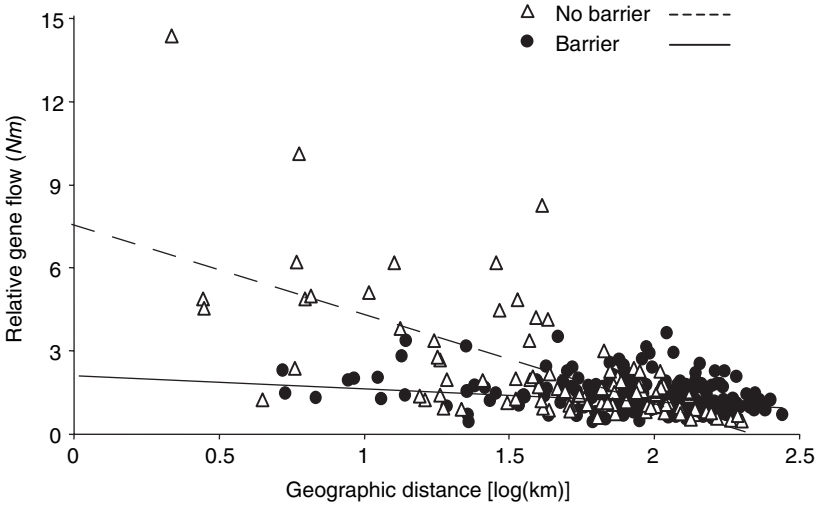

Figure 2 Pairwise population comparisons of migrants per generation (Nm, estimated from genetic distance F_{ST}) regressed on log(geographic distance in km). Comparisons are grouped by presence (dark circles) or absence (open triangles) of an intervening barrier. R^2 of pairs without barriers = 0.43, R^2 of pairs with barriers = 0.08. Regression lines are extended to cross the y-axis; difference in intercepts was used to calculate the 'barrier effect' (see text).

number of migrants (Whitlock & McCauley 1999). Under such a model Nm is correlated to F_{ST}, but in a nonlinear manner and thus we have used both metrics in our estimation. While our population is likely not in mutation-drift-migration equilibrium, Nm (and F_{ST}) can provide insights as to the relative levels of gene flow, especially when the rate of gene flow is larger than mutation (Slatkin 1993).

We employed partial Mantel tests over sequential geographical distance classes (0–15, 15–30, 30–60, 60–90, 90–120, 120–150 and > 150 km) (Legendre & Fortin 1989; Bjornstad *et al.* 1995; Dodd *et al.* 2002) to assess the partial correlation of barriers and geographical distance with Nm for each distance category. This allowed us to infer the spatial scale at which recent gene flow has occurred or has been disrupted by barriers. For a given distance category, interpopulation distances falling within that range were denoted as '1', all others as '0'. Similarly barriers were noted as '1' (present) or '0' (absent) for population comparisons within the given distance category. In this assessment, we excluded the Coxcomb Mountain population. Most of the bighorn in the Coxcomb Mountains were found to have immigrated from a nearby population, which was established by translocation from a distant population (Epps *et al.* 2005b). While the validity of estimates of type I error (here, falsely concluding that correlation of one independent matrix with the dependent matrix exists, because of correlation with a second independent matrix) in partial Mantel tests has been questioned (Raufaste & Rousset 2001; Rousset 2002), Castellano & Balletto (2002) argued that under even high levels of correlation between the independent matrices, partial Mantel tests closely approximate true type I error.

Finally, we employed the computer program SIMCOAL (Excoffier *et al.* 2000) to investigate if barriers could create a detectable increase in genetic distance between populations, given the time scale and data richness that apply to this study. Coalescent simulations were conducted under two different models, each simulating two adjacent populations 5 km apart. In the first model, we tested the effects of a recently constructed barrier by simulating two populations at mutation-drift-migration equilibrium except during the last seven generations (*c.* 42 years; Coltman *et al.* 2003), when Nm was set to zero. No such reduction in Nm was added to the second model. In each model 40 gene copies were sampled at each of 14 loci. SIMCOAL uses a pure stepwise migration model (in this case, without constraint on allele size), and requires the user to set migration rate m, effective population size N and mutation rate μ. SIMCOAL immediately multiplies these parameters to obtain Nm and θ, where $\theta = 4N\mu$. To obtain realistic values of Nm and θ for use in the model, we estimated $Nm = 6.2$ from the observed estimate of $F_{ST} = 0.039$ between a representative pair of mountain ranges, the Marble and South Bristol Mountains, that are separated by only 5 km with no intervening barrier. We estimated θ from the variance in allele size as $\theta = 2 \times$ (variance in allele size) (Wehrhahn 1975) for both of these mountain ranges ($\theta = 9.62$ and 8.32 respectively), and used the average of these values ($\theta = 8.97$) in our simulation. We also estimated θ from expected heterozygosity as $H_e = 1 - (1 + 2\theta)^{-1/2}$, giving an average of $\theta = 3.27$. For comparative purposes, we tested both of these measures of θ in our simulations, as well as $\theta = 1$. We varied values of Nm to include 2, 6.2 and 10. We calculated population pairwise F_{ST} between the two simulated populations for each simulation run using Arlequin (Schneider *et al.* 2000). For each parameter set, 1000 simulation runs from both models were compared to determine the average increase in F_{ST} because of barriers.

We compared this simulated average increase in F_{ST} because of barriers (for populations 5 km apart) to the observed increase in F_{ST} resulting from barriers for populations separated by this distance. We estimated the observed increase by regressing F_{ST} on log(geographic distance) for all population pairs with intervening barriers and for all population pairs without intervening barriers, and calculated the difference in the predicted F_{ST} values at 5 km using these two regression equations.

RESULTS

We obtained complete genotypes at all 14 microsatellite loci from 461 faecal and 47 blood or tissue samples. From our analyses of these 508 genotypes, we inferred that they represented a total of 397 individuals, yielding a mean sample size per population of 15 individuals (range 6–29, SD 5.9; Appendix S2). We identified 21 unique mtDNA haplotypes from 394 of these individuals; one haplotype had been previously described (GenBank no. AF076912, Boyce *et al.* 1999). New haplotype sequences were submitted to the GenBank database under the accession numbers AY903993–AY904012. Numbers of alleles and haplotypes per population, A, expected heterozygosity and other basic data are described in Appendix S2.

In the final microsatellite data set, we did not observe any case of allelic dropout among the consensus genotypes of the 111 samples that we identified as duplicates of previously sampled individuals. We found no evidence of linkage disequilibrium within populations after correcting for multiple comparisons.

The average rate of allelic dropout per locus per replicate for the faecal samples was estimated at 3.7%, while rate of occurrence of false alleles was estimated at 0.062%. Overall this yielded a final estimate of 0.022 genotypic errors per individual. Given an error rate of 0.022, in a sample set of *c.* 400 individuals typed at 14 loci, the expectation is approximately 10 single-locus errors in consensus genotypes.

Because this estimated error rate assumes that every sample is heterozygous at all loci, and that there were only two replicate PCR amplifications at each locus, this estimate of the genotype error rate is likely higher than the actual rate: most samples were successfully amplified three to four times. Assuming that genotype errors were randomly distributed with respect to population, this error rate was unlikely to bias our estimates of genetic diversity and divergence in a significant manner for the purposes of this study.

The 'barrier effect distance' was estimated at *c.* 40 km [$\Delta Nm = 5.05 = 3.177 \times \log(\text{'barrier effect' in km})$]. Genetic diversity was negatively correlated with both measures of population isolation ($\text{isolation}_{\text{distance}}$ and $\text{isolation}_{\text{distance+barriers}}$) (Fig. 3). However, using $\text{isolation}_{\text{distance+barriers}}$ significantly improved regression model fit for A (Table 1; Fig. 3), indicating that the presence of barriers reduced nuclear genetic diversity. The estimated decline in A for populations isolated by barriers from all three of the nearest populations was as high as 15%. Results for mtDNA haplotype diversity were more equivocal: although $\text{isolation}_{\text{distance+barriers}}$ had a better model fit than $\text{isolation}_{\text{distance+barriers}}$ as assessed by model

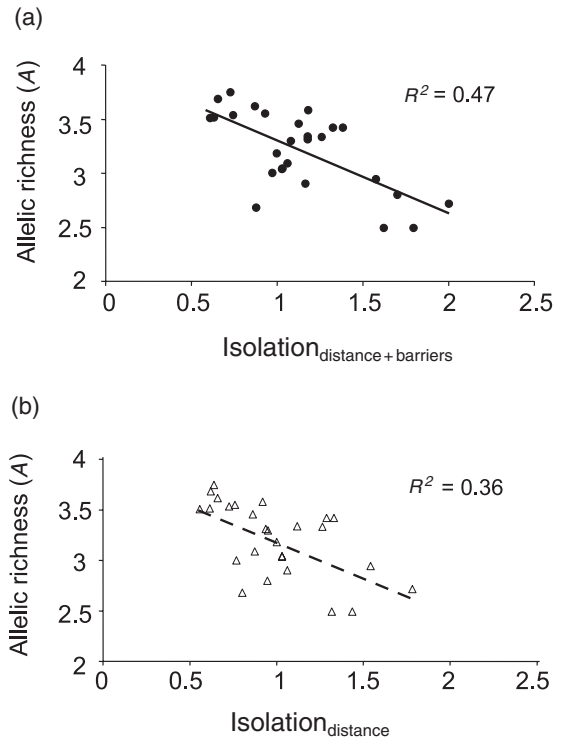

Figure 3 Regressions of allelic richness (A) on isolation as a function of distance and barriers (a) or distance alone (b). Isolation measures are based on log-transformed distances in km (see text).

F-statistic significance and R^2, and greater likelihood as assessed by AIC$_c$weight, the difference was not enough to clearly indicate that $\text{isolation}_{\text{distance+barriers}}$ was the best model (Table 1). Fits of both models for mtDNA haplotype diversity were poor ($R^2 < 0.20$), suggesting that neither model was adequate. Genetic diversity (nuclear and mitochondrial) was not correlated with population area or current estimated population size (Table 1).

The amount of gene flow among populations was strongly and negatively correlated with barriers at interpopulation distances of < 15 km (Mantel $r = -0.49$, $P = 0.0002$). When the effect of barriers was removed by partial correlation, Nm was strongly correlated among populations within 15 km (Mantel $r = 0.82$, $P = 0.0002$), weakly correlated among populations 15–30 km apart (Mantel $r = 0.16$, $P = 0.0448$), and not correlated among populations separated by greater distances. Plotting Nm as a function of distance also showed that Nm decreased sharply with distance for population pairs not separated by barriers (Fig. 2). Population pairs separated by barriers showed very low Nm values regardless of distance, suggesting that no exchange of individuals occurred across barriers (Fig. 2).

Partial correlations of pairwise F_{ST} values (genetic differentiation; Appendix S3) with barriers and distance showed a similar but weaker pattern. F_{ST} was positively correlated with the presence of barriers at interpopulation distances of < 15 km (Mantel $r = 0.168$, $P = 0.0220$) and 15–30 km (Mantel $r = 0.145$, $P = 0.0446$). F_{ST} was negatively correlated with the presence of populations within 15 km (Mantel $r = -0.444$, $P = 0.0002$), less strongly so at 15–30 km (Mantel $r = -0.174$, $P = 0.0264$), and not significantly correlated at greater distances. Because effects for both factors were detected in the first two distance classes, we also examined them across a 0–30-km distance class: F_{ST} was positively correlated with the presence of barriers (Mantel $r = 0.212$, $P = 0.0034$) and negatively correlated with the presence of populations within 30 km (Mantel $r = -0.441$, $P = 0.0002$).

Simulated datasets revealed that an increase in genetic distance (F_{ST}) because of barriers could be detected within the time frame of the age of the barriers in this study (*c.* 40 years). However, the increase in F_{ST} (0.012–0.018 depending on the parameter values used, Table 2) was not as large as the estimated increase in F_{ST} because of barriers for the actual study populations. The model of two simulated populations, 5 km apart with no intervening barrier, had an average F_{ST} ranging from 0.007 to 0.048 across the parameter set ($F_{ST} = 0.039$ between the study populations from which parameters were derived). Average F_{ST} between two simulated populations with a barrier present during the most recent seven generations increased for all parameter combinations; the increase did not appear to be greatly sensitive to the different values of Nm and θ

Table 1 Regression models of genetic diversity (corrected for sample size) as a function of human-made barriers, distance and other variables for $n = 27$ populations of desert bighorn sheep

Response variable	Model	*P*-value†	R^2‡	*k*§	ΔAIC_c	w_i¶
Allelic richness (corrected)	Log ($isolation_{distance+barriers}$)*	< 0.0001	0.47	3	0	0.88
	Log ($isolation_{distance}$)	0.0010	0.36	3	5.01	0.07
	Log ($isolation_{distance}$), population area	0.0031	0.38	4	6.96	0.03
	Log ($isolation_{distance}$), population size	0.0048	0.36	4	7.90	0.02
Number of mtDNA haplotypes (corrected)	$Isolation_{distance+barriers}$*	0.0388	0.16	3	0	0.63
	$Isolation_{distance}$*	0.0754	0.12	3	1.22	0.34
	$Isolation_{distance}$, population area, population size	0.3035	0.14	5	6.33	0.03

Model selection was performed using Akaike's Information Criterion (AIC)$_c$: models with lowest AIC_c values are best fit, but models within two ΔAIC_c units of the best model are considered equally explanatory. AIC weights (w_i) may be interpreted as the likelihood that the given model is the best of the candidate models (Burnham & Anderson 1998).
*Best-fit or competing model (within two AIC_c units).
†Significance of model *F*-statistic.
‡Fit of linear regression model.
§Number of predictor variables + 2 for calculating AIC_c.
¶AIC_c weight.

Table 2 Increases in average F_{ST} (with standard error) because of elimination of gene flow by a barrier for seven generations between two simulated populations (based on 1000 simulations)

θ	$Nm = 2$	$Nm = 6.2$	$Nm = 10$
1.00	0.015 (0.002)	0.012 (0.003)	0.013 (0.003)
3.27	0.018 (0.005)	0.015 (0.003)*	0.013 (0.003)
8.97	0.013 (0.004)	0.014 (0.002)†	0.012 (0.002)

*Nm calculated from observed F_{ST}, θ estimated from observed heterozygosity.
†Nm calculated from observed F_{ST}, θ estimated from variance in allele size.

that we employed (Table 2). However, the relative increase was sensitive to Nm and θ, in that low values of Nm increased average F_{ST} values between populations but not the difference caused by barriers. Estimated F_{ST} between the actual study populations, 5 km apart with an intervening barrier, increased from 0.046 to 0.113. This estimated increase was based on the regression equations of F_{ST} on distance for population pairs without barriers [$F_{ST} = -0.029 + 0.108 \times \log$(geographic distance in km)] and for population pairs with intervening barriers [$F_{ST} = 0.080 + 0.048 \times \log$(geographic distance in km)].

DISCUSSION

Nuclear genetic diversity of desert bighorn sheep populations was negatively correlated with the presence of human-made barriers that blocked dispersal to nearby populations (Table 1; Fig. 3). This finding strongly suggests that these barriers have reduced genetic diversity for many of these populations. We estimate from our results that nuclear genetic diversity in populations completely isolated by human-made barriers has declined as much as 15% in the *c.* 40 years since most barriers were erected. This estimate implies that the rate of loss of genetic diversity in populations isolated by barriers was *c.* 0.4% per year; if this rate is constant, some populations may lose up to 40% of their pre-barrier genetic diversity in the next 60 years. Results for mtDNA markers were consistent with these findings, but did not clearly support the reduction of mitochondrial genetic diversity because of barriers. The low correlation of mtDNA diversity with either distance and barriers may reflect very low dispersal rates for female bighorn sheep, as suggested by Festa-Bianchet (1991) and Jorgenson *et al.* (1997). More probably, the ambiguous results for mtDNA may reflect the stochasiticity inherent in one genetic locus (as represented by the mtDNA genome) when compared with the results derived from 14 microsatellite loci.

We believe that genetic diversity declined so rapidly after isolation because N_e of each population was likely very small. Therefore, unless diversity was maintained by gene flow from other populations, genetic drift quickly eliminated diversity. Our analyses of gene flow based on regression and partial correlation of Nm and F_{ST} with barriers and distance showed that, where present, human-made barriers have essentially eliminated dispersal (Fig. 2). The suppression of migration by barriers was most detectable within the distances at which high relative gene flow was most detectable, in this case, at < 15 km. Populations < 15 km from other populations maintained higher genetic diversity unless a human-made barrier intervened.

Finally, genetic simulations demonstrated that barriers constructed only 40 years ago could create a detectable increase in genetic distance between populations, although the increase in genetic distance in the simulations was not as great as that observed. This discrepancy may have resulted from a variety of factors. For one, these simple simulations considered only two populations. Actual populations experienced gene flow from other nearby populations, and probably experienced strong fluctuations in population sizes (perhaps caused by strong environmental stochasticity), founder effects, and other demographic events not included in the simulations that may have increased genetic distances. Thus parameter estimates (based on equilibrium conditions) for these simulations may not have been correct, although simulations with varied parameter estimates showed similar increases in genetic distance because of barriers. Historical census data (Torres *et al.* 1994), tiny population sizes and frequent recent extinctions of populations of desert bighorn sheep in California (Epps *et al.* 2004) suggest that fluctuations and founder effects have been common in the decades since the barriers have been constructed. Such metapopulation dynamics may further explain why barriers had such a strong effect on genetic diversity and genetic distance in only *c.* 40 years; this question bears further investigation with more realistic models. However, the detectable differences that our simple simulations yielded support our inference that observed patterns of genetic diversity could be due to the effects of human erected barriers (i.e. occur over such short-time frame).

Because our analyses rely on correlation of the presence of barriers with decreased genetic diversity and increased genetic distance, we cannot exclude the possibility that the genetic structure apparently created by barriers is an artifact of historical genetic structure. However, no other biogeographical explanation for such structure is readily apparent. While it is possible that roads may be constructed preferentially in flat areas or valleys between mountain ranges, nearly all of the populations considered are topographically isolated by flat areas, regardless of the presence of barriers (Fig. 1). Distance thus appears to be the prevailing natural barrier in this system, as evidenced by the strong correlation of genetic diversity and gene flow with distance, and was included explicitly in this analysis. Non-equilibrium conditions may have also affected estimates of genetic distance and other analyses. Despite this, the large number of populations considered and the consistent relationships between genetic diversity, genetic distance and the presence of barriers suggest that these findings are robust.

Our analyses point to the conclusion that human-made barriers may greatly reduce stability of the system as a whole: populations are small and re-colonization of extinct habitat patches is critical for metapopulation persistence (Hanski & Gilpin 1997; Gonzalez *et al.* 1998). Extinction risk for many desert bighorn sheep populations in California is high, and may sharply increase in the coming century because of climate warming (Epps *et al.* 2004). If movement corridors from climatically stable refugia (high-elevation ranges in this case) to more ephemeral patches are severed, re-colonization or demographic 'rescue' will be unlikely to occur. Moreover, connectivity is critical to maintain genetic diversity over the whole metapopulation. Even though strong genetic drift may rapidly remove genetic diversity from individual populations in a functioning metapopulation, this loss can be off-set by gene flow from other populations. However, if barriers disrupt gene flow and recolonization, genetic diversity may be lost very rapidly from the system as a whole (given that the total number of populations in this instance is not large). Thus barriers can have severe consequences both for demographic and genetic processes in metapopulations and may increase the danger of metapopulation extinction.

We recommend that consideration be given to ways to mitigate existing human-made barriers, and that any future construction of major highways in desert bighorn habitat should be designed to minimize disruption of connectivity. Drainage tunnels under interstate highways already exist in some areas (e.g. under Interstate 40 between the Marble and Granite mountains); while presumably large enough to allow traversal by bighorn sheep, these tunnels are within the fenced interstate corridor. Underpasses and overpasses have been used successfully to aid dispersal of carnivores and ungulates (Foster & Humphrey 1995; Gloyne & Clevenger 2001). Changes in fencing could allow access to tunnels while still preventing livestock or wildlife from entering the highway corridor itself. Overpasses could be another, perhaps more effective means of reestablishing connectivity for bighorn sheep, although the cost of such structures could be very high.

As the human population continues to expand, the need to maintain connectivity of natural populations is even greater. Rapid development of highways and other barriers has reduced and fragmented habitat for many species, while global climate change is increasing local extinction rates and forcing latitudinal or elevational shifts in species' distributions (Walther *et al.* 2002). Species-specific solutions to restoring habitat connectivity both in previously fragmented landscapes and relative to future development must be implemented.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online for this article from http://www.Blackwell-Synergy.com:

Appendix S1 (a) Zinc-finger gene primers and protocol, (b) microsatellite analysis protocols and references, and (c) mitochondrial DNA sequencing protocols.

Appendix S2 Sample sizes for analyses of microsatellite genotypes and mtDNA sequences, and basic genetic and geographical statistics for the 27 populations of desert bighorn sheep used in this study.

Appendix S3 F_{ST} values for all sampled populations, estimated from 14 microsatellite loci using GENEPOP.

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Appendix 1-a Zinc-finger gene primers and protocol.

We used the following primers, designed by R. Ramey, to screen for and remove weakly-amplifying extractions: ZFYf2 5'-3' TTA CTG AAT CGC CAC CTT TTG GC and ZFYr1 5'-3' CTG CAG ACC TAT ATT CGC AGT ACT (annealing temperature 57º; same experimental conditions employed for microsatellite analyses in Wehausen et al. (2004)).

Appendix 1-b Microsatellite analysis protocols and references.

Experimental conditions and references for 11 of the 14 dinucleotide microsatellite loci used in this study were described previously (Wehausen *et al.* 2004); we used the additional loci OarFCB128 and OarFCB266 (Buchanan & Crawford 1993) (annealing temperature 57º) and D5S2 (Steffen *et al.* 1993) (annealing temperature 55º). Amplification products were visualized using an ABI Prism™ 377 (Applied Biosystem Inc., Foster City, USA); alleles were designated using GeneScan™ (version 3.7, Applied Biosystem Inc., Foster City, USA) and Genotyper™ (version 3.7 NT, Applied Biosystem Inc., Foster City, USA).

Appendix 1-c Mitochondrial DNA sequencing protocols.

For mtDNA sequencing, we used ABI Prism™ 377 and 3730 sequencers (Applied Biosystems, Inc., Foster City, USA) and the following primers designed by R. Ramey: L15712 5'-3' AAC CTC CCT AAG ACT CAA GG and BETH 5'-3' ATG GCC CTG

AAG AAA GAA CC. We used 20 μL PCR reactions with the following reaction conditions: 1x PCR Buffer I (Applied BioSystems Inc., Foster City, USA), 0.16 mM dNTPs, 10 μg bovine serum albumin (New England BioLabs, Beverly, USA), 1.9 mM $MgCl_2$, 400 nM each primer, 0.8 units of Amplitaq Gold DNA polymerase (Applied BioSystems Inc., Foster City, USA), and 1 μL of extracted DNA. We used an initial heating cycle of 94º C for 7 minutes 30 seconds, followed by 35 cycles of 94º C for 60 seconds, 61º C for 70 seconds, and 72º C for 90 seconds. We cleaned PCR reactions using 0.2 units of shrimp alkaline phospatase (USB, Cleveland, USA) and 2 units of Exo I (New England Biolabs, Beverly, USA) to clean 1 μL of amplified DNA. We cycle-sequenced with BigDye™ v3.1 (Applied Biosystem Inc., Foster City, USA) following standard protocols.

References (Appendix 1)

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Appendix 2 Sample sizes for analyses of microsatellite genotypes and mtDNA sequences (if different, noted parenthetically) and basic genetic and geographic statistics for the 27 populations of desert bighorn sheep used in this study. $Isolation_{DISTANCE}$ is the harmonic mean of the distances to the nearest three populations; $Isolation_{DISTANCE+BARRIERS}$ adds the "barrier effect distance" of 40 km to inter-population distances if a barrier intervened (see text).

Population	[*] n	[†] H_e	Total alleles	[‡] A	[§] N_{HAP}	[¶] N_{HAP} (corrected)	$Isolation_{DISTANCE}$ (km)	$Isolation_{DISTANCE+BARRIERS}$ (km)
Clark	12	0.614	57	3.52	2	1.92	4.1	4.3
Clipper	16	0.647	54	3.31	4	3.30	8.6	15.1
Coxcomb	7	0.622	51	3.46	3	2.86	7.3	13.5
Cushenbury	15	0.489	38	2.49	1	1	20.8	62.6
Chemehuevi	9	0.571	51	2.94	1	1	34.8	37.8
Eagle-Buzzard Spr.	17	0.653	61	3.68	1	1	4.2	4.5
Eagle-Lost Palms	14	0.627	62	3.75	3	2.31	4.4	5.3
Granite	21	0.627	66	3.62	6	3.52	4.6	7.5
Hackberry	13	0.637	49	3.18	1	1	10.0	10.0
Iron	11	0.537	43	2.68	2	1.51	6.4	7.6

Cady	12	0.591	53	3.34	4	3.28	13.2	15.1
Little San Bernardino	12	0.626	57	3.58	3	2.34	8.3	15.2
Marble	29 (28)	0.644	61	3.55	3	1.77	5.8	8.5
Newberry	15	0.496	37	2.49	2	1.93	27.2	42.0
Old Dad	25	0.561	51	3.04	3	2.75	10.7	10.7
Indian Spring	12	0.475	48	2.90	3	2.06	11.5	14.7
Orocopia	18	0.568	47	3.00	3	1.97	5.9	9.4
Old Woman	26	0.512	54	3.04	3	2.39	10.8	10.8
Piute Range	13	0.627	55	3.42	3	2.68	21.3	21.3
Providence	20	0.628	59	3.51	5	3.37	3.6	4.1
Queen	11	0.594	55	3.42	3	2.49	19.4	24.4
Riverside Granite	10 (8)	0.609	47	3.09	2	2.00	7.5	11.5
South Bristol	14	0.599	51	3.29	2	1.98	8.9	12.1

San Gorgonio	17	0.539	44	2.80	1	1	8.9	50.3
San Gabriel	6	0.549	38	2.71	1	1	60.6	101.8
Turtle	14	0.635	54	3.33	2	1.43	18.3	18.3
Wood	10	0.622	55	3.53	3	2.49	5.3	5.6

[*] number of individuals sampled per population

[†] expected heterozygosity

[‡] allelic richness corrected for variation in sample size

[§] number of mtDNA haplotypes detected

[¶] number of mtDNA haplotypes corrected for variation in sample size

Appendix 3 F_{ST} values for all sampled populations, estimated from 14 microsatellite loci using GENEPOP. All values were significantly different ($p<0.05$). Population names are abbreviated but are presented in the same order as in Appendix 2.

	CL	CO	CU	CV	EABZ	EALP	GR	HA	IR	KD	LS	MA	NE	OD	OE	OR	OW	PI	PR	QU	RG	SB	SG	SL	TU	WO
CK	0.13	0.14	0.29	0.16	0.10	0.11	0.09	0.11	0.21	0.15	0.13	0.14	0.25	0.15	0.20	0.15	0.18	0.09	0.12	0.13	0.12	0.15	0.21	0.15	0.14	0.10
CL		0.15	0.24	0.18	0.10	0.10	0.08	0.14	0.21	0.13	0.13	0.05	0.20	0.15	0.19	0.18	0.17	0.12	0.11	0.15	0.11	0.07	0.20	0.20	0.16	0.13
CO			0.27	0.16	0.11	0.07	0.07	0.10	0.16	0.10	0.10	0.14	0.26	0.06	0.20	0.19	0.12	0.11	0.10	0.09	0.10	0.15	0.16	0.22	0.16	0.06
CU				0.35	0.18	0.18	0.20	0.31	0.26	0.28	0.20	0.25	0.37	0.27	0.32	0.29	0.22	0.26	0.27	0.23	0.22	0.24	0.07	0.37	0.31	0.26
CV					0.21	0.21	0.12	0.15	0.29	0.18	0.24	0.18	0.33	0.18	0.24	0.25	0.22	0.16	0.19	0.22	0.20	0.19	0.27	0.26	0.22	0.14
EABZ						0.02	0.07	0.13	0.17	0.14	0.04	0.13	0.25	0.15	0.19	0.15	0.13	0.10	0.09	0.06	0.07	0.12	0.12	0.21	0.13	0.12
EALP							0.06	0.12	0.13	0.13	0.04	0.12	0.23	0.11	0.18	0.16	0.10	0.10	0.08	0.03	0.07	0.10	0.12	0.20	0.12	0.09
GR								0.06	0.17	0.11	0.08	0.10	0.18	0.09	0.13	0.17	0.12	0.08	0.05	0.11	0.08	0.11	0.13	0.17	0.13	0.05
HA									0.21	0.14	0.13	0.13	0.21	0.14	0.19	0.22	0.18	0.08	0.06	0.16	0.17	0.16	0.21	0.17	0.18	0.02
IR										0.24	0.18	0.21	0.32	0.19	0.29	0.29	0.05	0.19	0.18	0.14	0.17	0.23	0.19	0.21	0.21	0.19
KD											0.15	0.09	0.26	0.11	0.10	0.17	0.17	0.12	0.15	0.16	0.12	0.12	0.20	0.21	0.18	0.12
LS												0.14	0.24	0.16	0.21	0.17	0.13	0.12	0.12	0.05	0.11	0.14	0.15	0.22	0.13	0.13
MA													0.14	0.16	0.18	0.17	0.15	0.10	0.11	0.16	0.12	0.04	0.19	0.20	0.15	0.13
NE														0.27	0.35	0.30	0.26	0.15	0.19	0.25	0.28	0.19	0.27	0.32	0.25	0.24
OD															0.10	0.23	0.16	0.13	0.11	0.17	0.15	0.15	0.18	0.21	0.20	0.11
OE																0.26	0.20	0.19	0.18	0.22	0.21	0.20	0.24	0.24	0.26	0.17
OR																	0.23	0.20	0.22	0.13	0.15	0.16	0.24	0.25	0.20	0.22
OW																		0.14	0.15	0.10	0.11	0.17	0.16	0.18	0.15	0.16
PI																			0.07	0.13	0.12	0.12	0.16	0.17	0.09	0.05
PR																				0.13	0.15	0.14	0.17	0.18	0.15	0.05
QU																					0.09	0.14	0.16	0.21	0.15	0.15
RG																						0.13	0.16	0.22	0.13	0.13
SB																							0.20	0.23	0.18	0.14
SG																								0.27	0.21	0.18
SL																									0.22	0.18
TU																										0.16