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Tools and Technology Article

Using Genetic Tools to Track Desert Bighorn Sheep Colonizations

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ABSTRACT Understanding colonization is vital for managing fragmented populations. We employed mitochondrial DNA haplotypes and 14 microsatellite (nuclear DNA) markers to infer the origins of newly established populations of desert bighorn sheep (*Ovis canadensis nelsoni*) and to assess loss of genetic diversity during natural colonizations. We used haplotype distribution, *F*-statistics, Bayesian population clustering, and assignment tests to infer source populations for 3 recent colonies and identified a previously undetected colonization from multiple source populations. Allelic richness declined in 3 of 4 colonies in comparison to the primary source populations, but not as much as has been reported for translocated populations. Heterozygosity declined in only one colony. We also demonstrated that both native and translocated desert bighorn sheep have naturally recolonized empty habitats and suggest that colonization may partially offset population extinction in the region as long as connectivity is maintained. Genetic techniques and mitochondrial DNA haplotypes we described will allow managers to determine the origins of future colonizations by bighorn sheep in California, USA, and prioritize protection of linkages between known sources and colonies.

KEY WORDS assignment test, California, colonization, metapopulation, microsatellites, mitochondrial DNA, *Ovis canadensis*, translocation.

Colonization of empty habitat is required to offset high population extinction rates for species with fragmented distributions (Levins 1970). Understanding colonization could help wildlife managers identify and protect key linkages between habitat patches, prioritize translocations when natural colonization rates are thought to be inadequate, predict range expansions, and respond appropriately to newly discovered populations of unknown origins. Colonization processes also can affect loss or retention of genetic diversity, which has been linked to individual fitness and population performance (Vila et al. 2003, Hogg et al. 2006). However, studying colonization usually requires either repeated surveys in all potential habitat patches or long-term monitoring of many individuals (Ims and Yoccoz 1997).

Population genetic data offer alternative means to track or identify recent colonizations (e.g., Eldridge et al. 2001, Scribner et al. 2003, Latch et al. 2006*b*). Emigration of a few individuals to new habitat results in a founder event that is analogous to a population bottleneck and, therefore, is predicted to affect genetic structure and diversity in 2 ways. First, because of the underrepresentation of rarer alleles in emigrants, genetic diversity is expected to be lower in the colony than in the source population (Nei et al. 1975). Second, although allele frequencies in colony and source(s) are expected to diverge after a founder event (e.g., Mock et al. 2004, Hawley et al. 2006), the identity and frequency of alleles in a recent colony should be more similar to those in the source population(s) than to other nearby populations. The largest changes in allele frequencies and genetic diversity are expected when there are few founders, the colony remains small, and if there is no subsequent gene flow between colony and source (Nei et al. 1975, Keller et al. 2001). Those changes are also influenced by time since the founder event (see Cornuet and Luikart 1996). Changes in genetic structure and loss of genetic diversity resulting from population reintroductions or translocations (e.g., Mock et al. 2004, Whittaker et al. 2004), invasions (e.g., Hufbauer et al. 2004, Hawley et al. 2006), or rare long-distance natural recolonizations (e.g., Onorato et al. 2004, Hedmark and Ellegren 2007) have been well-described. Effects of local-scale colonizations on genetic structure and diversity in a metapopulation may be less predictable because the size of the founding population is rarely known and gene flow between source and colony may continue after colonization.

Bighorn sheep favor mountainous habitat that is often naturally discontinuous, resulting in natural metapopulations (Schwartz et al. 1986; Bleich et al. 1990, 1996; Epps et al. 2003). In the 19th and 20th centuries bighorn sheep suffered dramatic range reductions, many of which were attributed to disease and human exploitation (Buechner 1960). In the California, USA, deserts, however, extinctions were a more recent phenomenon of the mid–20th century (Wehausen et al. 1987, Wehausen 1999, Epps et al. 2004). In part because colonization was considered unlikely, bighorn sheep have been translocated extensively throughout the western United States (Ramey 1993). However, unaided colonization of empty habitat patches has now been well-documented (Bleich et al. 1996, Singer et al. 2000,

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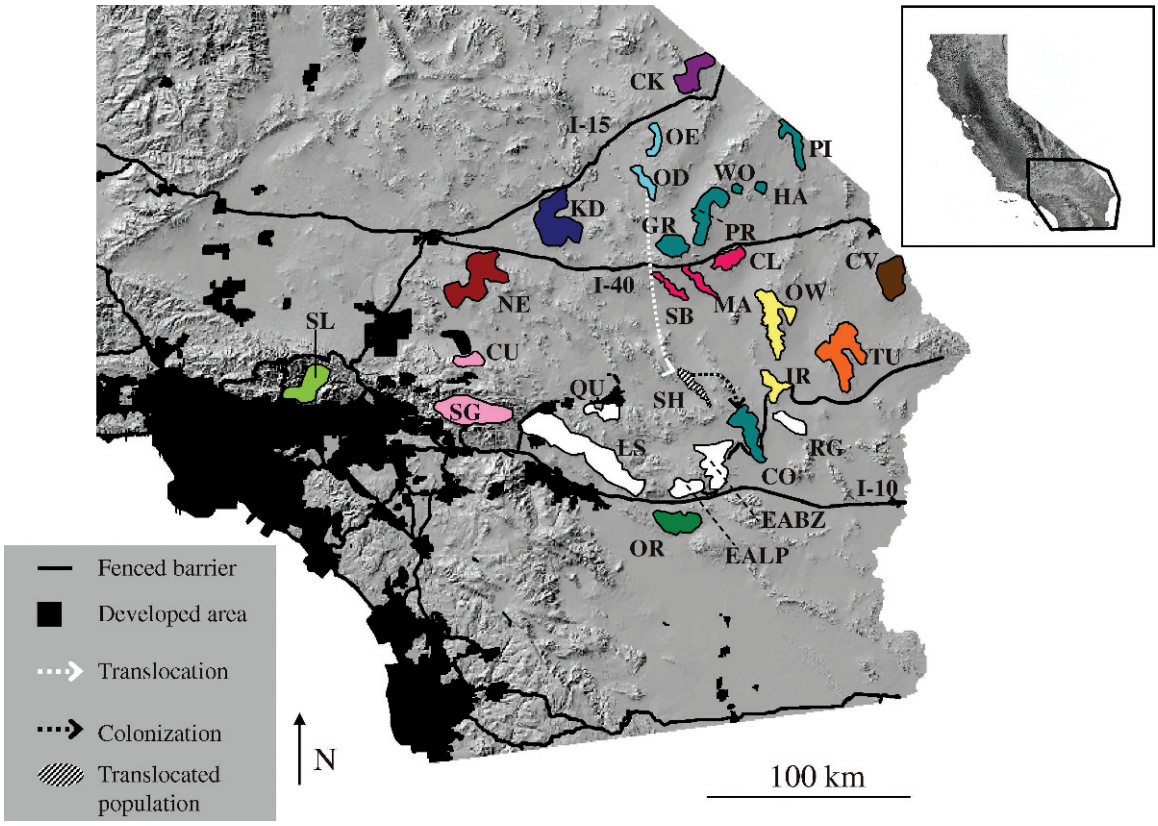

Figure 1. Relief map of southeastern California, USA, showing the 27 populations of desert bighorn sheep we sampled in 2000–2004, as well as the translocated population in the Sheephole Mountains (SH). Population polygon coloring represents results of BAPS clustering analysis; we clustered like-colored populations by genetic similarity, indicative of recent or current gene flow. We defined population identification codes in Appendix A; human-made dispersal barriers including fenced highways, fenced canals, and urban areas are mapped in black with interstate highways indicated as, for example, "I-15." We inferred colonization of the Coxcomb Range (CO; dashed black arrow) from SH because CO individuals were assigned genetically to the Old Dad (OD) population. Old Dad was the source of the bighorn sheep translocated to SH (dashed white arrow).

Epps et al. 2003). We define colonization as emigration of individuals of both sexes to an empty habitat patch, with subsequent reproduction. We examined recent colonizations by desert bighorn sheep (*Ovis canadensis nelsoni*) in California to determine 1) whether source populations could be confidently identified using standard tests for genetic structure and population assignment, and 2) whether significant reductions in genetic diversity (i.e., allelic richness) and heterozygosity occurred during natural colonizations.

STUDY AREA

We conducted our study in the Mojave and Sonoran Desert regions of California (Fig. 1), where desert bighorn sheep typically inhabited small mountain ranges isolated by flat desert with little water and limited forage. More than 50 native and reintroduced populations totaled approximately 4,200 bighorn sheep (*O. c. nelsoni*), but about half of those populations contained <50 individuals (Epps et al. 2003), making them vulnerable to genetic drift and loss of genetic diversity. Gene flow among those populations was affected by distance, topography, and human-made barriers such as fenced interstate highways (Epps et al. 2005, 2007). Due to frequent extinction of bighorn sheep populations in the 20th century, uninhabited mountain ranges occur throughout the study area (Wehausen et al. 1987, Wehausen 1999).

Although population monitoring often was sporadic (Wehausen 1999), radiotelemetry and population surveys identified 3 recent colonizations (or recolonizations). The South Bristol Mountains (SB; Fig. 1) were uninhabited (Torres et al. 1994) until 3 females radiocollared in the nearby (5 km) Marble Mountains (MA; Fig. 1) traveled to SB in 1993. By the late 1990s, a small but rapidly increasing population was established; a 2007 survey resulted in a mark–resight estimate of 68 individuals (J. D. Wehausen, White Mountain Research Station, personal communication). The second colonization occurred in the Iron Mountains (IR; Fig. 1). Observations at the sole known water source in IR indicated no resident sheep in 1993 (G. Sudmeier, Society for the Conservation of Bighorn Sheep, personal communication) but we observed males, females, and juveniles in 2001–2003. Historical evidence of movement by male and female bighorn sheep between the Old Woman Mountains (OW; Fig. 1) and IR, as well as movements between those ranges by radiocollared males, suggested OW was the likely source (Bleich et al. 1990; A. Pauli, California Department of Fish and Game, personal communication). The third apparent colonization occurred when emigrants from an unknown source founded the

Cushenbury population (CU; Fig. 1) in the 1980s (J. Davis, California Department of Fish and Game, personal communication), which at the time of our study was estimated at 25–50 individuals (Epps et al. 2003).

METHODS

Genetic Data Collection

We used previously published microsatellite genotypes and mitochondrial DNA (mtDNA) sequence data for 397 individuals from 27 populations of desert bighorn sheep in southeastern California (Fig. 1), collected from fecal and blood samples obtained during 2000–2004 (Epps et al. 2005). We restricted analyses to unique individuals that were successfully genotyped at all 14 microsatellite loci; 515 nucleotides near the beginning of the mtDNA control region were sequenced for 394 of these samples. Mean sample size per population was 15 individuals (range = 6–29).

Where sex identification was necessary, we determined sex of each individual sampled using the SE47 and SE48 sex identification primers (Yamamoto et al. 2002), which amplify fragments of different sizes on the X and Y chromosomes. We used 20 μL PCR (Polymerase Chain Reaction) with the following reaction conditions: 1× PCR Buffer I (Applied BioSystems, Foster City, CA), 0.16 mM dNTPs, 10 μg bovine serum albumin (New England BioLabs, Ipswich, MA), 2.25 mM $MgCl_2$, 80 nM each primer, 0.7 units of Amplitaq Gold™ DNA polymerase (Applied BioSystems), and 1 μL of extracted DNA. We used an initial heating cycle of 95° C for 7 minutes and 30 seconds, followed by 40 cycles of 95° C for 30 seconds, 54° C for 45 seconds, and 72° C for 30 seconds. We visualized the SE47 and SE48 amplification products on 2% agarose gels, prestained with ethidium bromide. We repeated amplifications until we observed the male-specific PCR fragment or we observed the single female band in 3 replicates.

Analyses

We applied common analytical techniques for describing genetic structure and diversity among populations to confirm (SB and IR) or infer (CU and other) source populations for recent colonizations and to test for population bottlenecks and decreased genetic diversity in colonies. Because most gene flow in this system occurred between populations <15 km apart (Epps et al. 2005), we evaluated potential source populations <30 km from each colonized population. To infer the source of females for each colony, we mapped distribution of mtDNA haplotypes (maternally inherited) in potential source populations. Female movements probably limited colonization because female bighorn are more philopatric than males (Festa-Bianchet 1986, Singer et al. 2000).

We evaluated whether genetic distance (F_{ST}; Wright 1921, Weir and Cockerham 1984) was significantly lower between colony and source in comparison to other nearby populations. Interpreting gene flow from F_{ST} is problematic unless populations are assumed to be in drift–migration–mutation equilibrium, which is unlikely in recent colonizations, but F_{ST} provides a useful relative estimate of population similarity (Neigel 2002). We used FSTAT (Goudet 1995) to calculate F_{ST} with bootstrap 95% confidence intervals from the microsatellite data between all population pairs. We also estimated F_{ST} from the mtDNA sequence variation and haplotype frequency (ARLEQUIN Version 3.11, http://cmpg.unibe.ch/software/arlequin3/; accessed 30 Jun 2008).

To distinguish clusters of populations linked by high gene flow, we grouped all 27 study populations by genetic similarity using Bayesian clustering methods employed by BAPS (Corander et al. 2003). We set burn-in time to 10,000, chain length to 50,000, thinning to 5, and ensured these values were sufficient to achieve convergence of estimates (Corander et al. 2003). We reported only clusters with posterior probabilities >0.95.

Individual-based assignment tests such as STRUCTURE (Pritchard et al. 2000) are often used to identify migrants or determine population origins (e.g., Mock et al. 2004). We used STRUCTURE to evaluate whether colonies had single or multiple origins. We combined all individuals from all 27 populations into one data set with no information on sample origin and estimated the likely number of clusters (k) of genetically similar individuals by running 10 simulations for each k from 1 and 30, using a burn-in of 500,000 chains followed by 1,000,000 chains for each run. We assumed that individuals were of admixed ancestry and allele frequencies were correlated (λ set at 1), and we allowed STRUCTURE to infer the degree of admixture. We identified the best value of k (k_{best}) as that where the second-order rate of change in the log-likelihood values for different k was maximized (Evanno et al. 2005). Program STRUCTURE fractionally assigns each individual to each cluster based on an assignment index (q) that sums to 1 across all clusters; we assigned individuals to the single most likely cluster based on the maximum value of q (q_{max}) estimated at k_{best} and defined individual assignments at $q_{max} > 0.5$ as high confidence.

Methods that assign individuals to a priori populations (e.g., mountain ranges) with high gene flow may split assignment probabilities among those populations. Alternatively, methods that assign individuals to clusters determined post hoc from genetic structure (e.g., STRUCTURE) may be hard to interpret or evaluate statistically, especially if assignment indices are low. To address both of those problems, we used GENECLASS2 (Piry et al. 2004) to assign 1) recently colonized populations to potential source populations (all other populations sampled) by ranked likelihood scores using the Bayesian classification method of Rannala and Mountain (1997) and an assignment threshold of 0.01, and 2) individuals from recently colonized populations to potential source populations (all other populations sampled) based on ranked likelihood scores. We then interpreted assignments to individual populations in the context of population clusters with high gene flow as identified by BAPS.

After identifying the most likely sources for each colony, we tested whether genetic diversity declined sharply during natural colonizations. We estimated allelic richness (A; average allelic diversity corrected for differences in sample size) at each locus for source and colony with FSTAT (Goudet 1995) and estimated unbiased heterozygosity (H_e; Nei 1987). After examining distribution of differences for normality, we used a 1-tailed paired sample t-test across loci to determine whether A and H_e in each colony were lower than in the respective sources (Zar 1999). For populations with clear evidence of mixed origins (see Results), we tested for differences in A and H_e via 2-tailed paired sample t-tests. We checked for population bottlenecks by testing for shifted modes in allele frequencies in each population (Luikart et al. 1998) using BOTTLENECK (Cornuet and Luikart 1996) because Mock et al. (2004) found that test to be most effective in detecting founder events.

RESULTS

We detected 19 mtDNA haplotypes in 27 populations (Appendix A; GenBank accession no. AY903993-AY904012, AF076912). The SB and IR colonies contained a subset of mtDNA haplotypes detected in their respective source populations (MA and OW; Appendix A). However, NE (>80 km W of SB; Fig. 1) also contained the 2 haplotypes found in SB (Appendix A). The CU colony (unknown source) contained only one haplotype (N), also found in SG, and found elsewhere only in the more distant QU, LS, EALP, and RG complex of populations (Appendix A; Fig. 1). We detected none of the NE haplotypes in CU, implying that SG was the most likely source of colonizing females. Genetic distance values calculated from mtDNA values accurately linked IR–OW and supported CU–SG (Table 1) but conflicted with other data in one case: $F_{ST\text{-}mtDNA} = 0$ for NE–SB, whereas $F_{ST\text{-}mtDNA} = 0.11$ for MA–SB (the likely source–colony pair based on radiotelemetry and microsatellite analyses; Table 1). Genetic distance estimates (F_{ST}) from microsatellite data generally corroborated known source–colony pairs, although wide confidence intervals precluded confident distinction of the source population for SB (Table 1). Despite identical mtDNA haplotypes in NE and SB, microsatellite markers did not support NE as a population of origin (Table 1). Genetic distance between CU and NE was 5 times higher than that between CU and SG, implying that male as well as female colonizers originated in SG (Table 1).

Bayesian population (not individual) clustering via BAPS from the microsatellite data resulted in 13 population clusters (Fig. 1). Although BAPS may overestimate cluster number (Latch et al. 2006*a*), the observed number of clusters was less than determined by STRUCTURE (below). Program BAPS grouped MA with SB and grouped OW with IR (Fig. 1). The CU population was linked to SG rather than NE. A population previously considered to be native (CO) was grouped with PR, HA, WO, and PI >95 km north rather than with other nearby populations (Fig. 1).

Table 1. Genetic distance (F_{ST}) values for microsatellite (with 95% CIs) and mitochondrial DNA (mtDNA) markers for 4 recently colonized populations and potential source populations of desert bighorn sheep we sampled in 2000–2004 in California, USA. See Appendix A for explanation of population codes.

Population pair	F_{ST} (microsatellite markers)			F_{ST} (mtDNA)
	Point estimate	95% CI (lower lim)	95% CI (upper lim)	
SB–MA[a]	0.039	0.019	0.061	0.112
SB–GR	0.111	0.057	0.171	0.336
SB–NE	0.189	0.110	0.274	−0.005
SB–KD	0.118	0.079	0.157	0.802
SB–OD	0.152	0.091	0.220	0.767
SB–PR	0.140	0.093	0.196	0.384
SB–CL	0.069	0.046	0.097	0.589
IR–OW[a]	0.048	0.023	0.075	0.0346
IR–TU	0.212	0.161	0.273	0.946
IR–RG	0.212	0.105	0.237	0.872
IR–CO	0.157	0.094	0.214	0.596
CU–SG[a]	0.069	0.035	0.102	0
CU–SL	0.374	0.274	0.471	1
CU–LS	0.197	0.133	0.260	0.768
CU–QU	0.233	0.169	0.301	0.387
CU–NE	0.372	0.276	0.459	0.850
CO–OD[a]	0.059	0.026	0.096	0.320
CO–EABZ	0.110	0.042	0.191	0.686
CO–IR	0.157	0.092	0.214	0.596
CO–RG	0.103	0.051	0.160	0.470

[a] Known or inferred comparison between source population and colony.

Using the Evanno et al. (2005) method for identifying cluster number with STRUCTURE, all 397 individuals were grouped into 14 genetic clusters ($k_{best} = 14$) from the microsatellite data. Individual assignments across replicate runs at same k were consistent, although q for each individual varied slightly; therefore, we present only the results of the first run at $k = 14$. Most individuals were grouped in clusters that matched source–colony pairings determined by other methods (Appendix B). Previously known colonies appeared to be of single origin with one possible exception: 13 of 14 individuals from SB were assigned to the same cluster as 23 of 29 individuals from MA (cluster c5; Appendix B), but the 14th individual, determined via SE47 and SE48 to be female, was assigned at low confidence ($q < 0.5$) to cluster c4, which included mostly individuals from other populations to the north (Appendix B; Fig. 1). All 11 individuals from the IR were assigned to cluster c8 at high confidence ($q > 0.5$), which also included 23 of 26 individuals from the OW population (22 at $q > 0.5$) but only one individual from TU ($q > 0.5$) and none from the CO, EABZ, and RG populations. Finally, all 15 of the CU individuals were assigned at $q > 0.5$ to cluster c9, to which none of the NE but all 17 of the SG individuals were also assigned at $q > 0.5$ (Appendix B).

Because of the counterintuitive clustering of CO with populations >95 km away by BAPS, we also examined individual assignments for CO after determining their sex with SE47 and SE48. Four males and one female were assigned (4 of 5 at $q > 0.5$) to the same cluster as 25 bighorn sheep sampled at OD (c4; Appendix B); those 5 sheep also had OD-type mtDNA haplotypes D or I (Appendix A).

Table 2. Sample size (n), differences in average allelic richness at 14 loci (A, corrected for the smaller sample size within each comparison) and average unbiased heterozygosity (H_e) as inferred from 1-tailed Wilcoxon paired-sample tests (except where noted), and shifted mode in allele frequencies test for population bottlenecks in source populations and colonies of desert bighorn sheep in California, USA, 2000–2004.

	n		A		H_e		Bottleneck	
Source–colony	Source	Colony	Source	Colony	Source	Colony	Source	Colony
MA–SB	27	14	4.1	3.6*	0.65	0.60	yes	yes
OW–IR	26	11	3.5	3.1*	0.51	0.46	no	no
SG–CU	17	15	3.1	2.7*	0.54	0.45*	no	yes
OD–CO	25	7	3.1	3.6[a]	0.52	0.58[a]	no	no[b]
EABZ–CO	17	7	3.8	3.6[a]	0.65	0.58[a]	no	no[b]

[a] 2-tailed Wilcoxon paired-sample test.
[b] Sample size below recommended min. of 10 individuals.
* $P < 0.05$.

The remaining 2 males were assigned at $q > 0.5$ to the same cluster as many of the bighorn sheep from the nearby EABZ, EALP, LS, QU, and RG populations (c12; Appendix B); those 2 individuals had mtDNA haplotype F, which was commonly found in those nearby populations but unknown at OD (Appendix A).

At the population level, GENECLASS2 grouped SB with MA, IR with OW, and CU with SG at likelihood scores of 100%. CO was grouped with GR (a population N of Interstate 40 and connected to OD with moderate gene flow; likelihood score = 91%) and OD (source of the translocated individuals in the SH population N of CO, likelihood score = 9%). At the individual level, GENECLASS2 assigned 12 of 14 individuals from SB to MA at likelihood scores >96%, one to CL (61%) and MA (39%), and the same female distinguished by STRUCTURE to OD north of Interstate 40 (90%). Ten of 11 individuals from IR were assigned to OW at scores >95%, and the 11th was assigned to OW at 51% with remaining assignment score percentage apportioned among the closely linked EALP, EABZ, LS, and QU populations (Fig. 1). All 15 individuals from CU were assigned to SG (13 at >99%, 1 at 93%, and 1 at 89%). Lastly, the 2 male individuals in CO with Haplotype F were assigned to QU with scores >99% (part of a BAPS cluster including the more likely EABZ; Fig. 1), whereas the other 5 individuals with OD-type mtDNA were assigned to OD (3 at >96%, 1 at 93%) and GR (1 at 89%).

In comparison with each inferred source, allelic richness (A) was lower for all 3 colonies primarily of single origin (Table 2; MA–SB $t_{1,13} = 3.10$, $P = 0.004$; OW–IR $t_{1,13} = 1.83$, $P = 0.045$; SG–CU $t_{1,13} = 2.06$, $P = 0.030$). Estimates of A in CO did not differ from OD (Table 2; $t_{2,13} = 1.79$, $P = 0.097$) or EABZ (Table 2; $t_{2,13} = 0.58$, $P = 0.284$). Heterozygosity in CU was 17% lower than in SG (Table 2; SG–CU $t_{1,13} = 3.15$, $P = 0.004$) but H_e did not decline for any other single-origin colony (Table 2; MA–SB $t_{1,13} = 1.48$, $P = 0.081$; OW–IR $t_{1,13} = 1.35$, $P = 0.100$). Estimated H_e for CO did not differ from OD (Table 2; $t_{2,13} = 1.05$, $P = 0.273$) or EABZ ($t_{2,13} = 1.51$, $P = 0.170$). We detected shifted modes in distribution of allele frequencies, indicative of recent population bottlenecks (Luikart et al. 1998), in MA and SB and CU but not SG. We did not detect shifted modes in IR, OW, CO, OD, or EABZ, although the sample size for CO was less than the recommended minimum (Table 2).

DISCUSSION

As expected given the philopatric and social nature of female bighorn sheep in particular (Festa-Bianchet 1986, Singer et al. 2000), the 3 previously known colonies (SB, IR, and CU) appear to have originated primarily from single source populations (MA, OW, and SG). Nearly all analyses agreed, although we detected 2 ambiguities: 2 possible source populations for SB had identical mtDNA haplotypes (Fig. 1; Appendix A), and one female in SB was assigned by STRUCTURE and GENECLASS2 to populations north of Interstate 40 rather than MA. Because that assignment was not at high confidence, that individual could be, rather than a migrant, the offspring of a migrant.

We also identified a possible cryptic colonization (CO; Fig. 1) with males originating in multiple source populations. Population clustering (BAPS) demonstrated that bighorn in CO were closer genetically to populations north of Interstate 40 (Fig. 1). Population CO is near the SH population, which was reestablished or augmented by translocation of desert bighorn sheep from population OD in 1984 and 1985 (Bleich et al. 1990, 1996). The F_{ST} estimate between CO and OD was 50% lower than the lowest estimate between CO and any other population (Table 1). Because the 1 female and 4 of 6 males sampled in CO had OD-type mtDNA haplotypes that could only have originated in SH, and because those same individuals were also assigned using nuclear DNA markers by both STRUCTURE and GENECLASS2 to OD or other distant northern populations, we hypothesize that females and males from SH recently recolonized CO after an unobserved extinction and were then joined by males from other nearby populations. Although neither mtDNA data, F_{ST} values, nor population assignments clearly indicated whether the 2 males with local haplotypes originated in EABZ, RG, or even QU to the west of EABZ (Table 1; Appendices A, B), the close proximity of the EABZ to CO and the presence of a fenced canal between the CO and RG imply that EABZ was the likely origin (Fig. 1). Because mtDNA and nuclear DNA assignments matched, little interbreeding appears to have occurred yet between the SH

(OD-derived) individuals and EALP–EABZ–RG-derived individuals present in CO.

The genetic structure and loss of genetic diversity that we detected for these colonizations demonstrate that bighorn sheep of both sexes will move substantial distances across unsuitable habitat, but only occasionally (Tables 1, 2). Although founding population sizes and the degree of subsequent gene flow between colony and source are still unclear, decreased A in the colonies (Table 2) implied that founder effects occurred and, thus, the size of the founding population was small. However, we did not observe declines in H_e except in CU (Table 2). Studies of translocated populations and long-distance colonizations have typically detected declines in A (e.g., Mock et al. 2004) but not always in H_e (e.g., Hicks et al. 2007), especially when founder numbers are high (e.g., Hufbauer et al. 2004). Expected heterozygosity is predicted to decline more slowly than A after a bottleneck, particularly if the colony or bottlenecked population grows rapidly (Allendorf 1986).

Direct comparisons of genetic diversity between source and colony were more informative than results of the bottleneck test; although we detected a bottleneck in CU but not SG, as might be expected after a founder event, we did not detect a bottleneck in IR or OW. Because we detected a bottleneck in MA, it is unclear whether the bottleneck detected in SB resulted from the founder event or reflects the bottleneck in the source population. Divergence (Table 1) and loss of genetic diversity (Table 2) was greatest in the SG–CU colonization, which also occurred over the greatest distance. Thus, ongoing gene flow may be an important mechanism for maintaining higher genetic diversity in the other less isolated colonies (i.e., OW–IR and MA–SB). For instance, radiocollared males made repeated movements between IR and OW during monitoring in 2001–2003, but no radiocollared individual in CU has returned to SG (J. Davis, personal communication).

Employing multiple analytical approaches strengthened inferences about source populations. Although F_{ST} estimates from mtDNA could not always determine population of origin (Table 1), mapping mtDNA haplotypes provided useful inferences on female dispersal and may provide sufficient resolution if strong genetic structure is suspected (e.g., Latch et al. 2006*b*). Comparing F_{ST} estimates from microsatellite markers identified the same source populations as other analyses but did not completely exclude one nearby nonsource population (Table 1; SB–GR) and did not distinguish multiple source populations for CO. Population clustering methods using BAPS (Fig. 1) and GENECLASS2 demonstrated isolating effects of both distance and human-made dispersal barriers such as fenced canals, interstate highways, and urban areas (Fig. 1) and identified the cryptic colonization of CO from SH.

Individual-based assignment tests (STRUCTURE, GENECLASS2) were useful for evaluating whether colonies had multiple origins but are difficult to summarize and interpret for large data sets. Counterintuitive results are common, such as an assignment at $q > 0.5$ for one individual in OW to the same cluster as SL (Appendix B, c3), which is >250 km distant. Therefore, we stress that interpreting assignment tests for individual animals requires great caution. Nonsensical assignments may result from homologous mutations, genotyping errors, or poor ability to distinguish clusters among areas of high gene flow (e.g., Worley et al. 2004). We had greater confidence in assignments of CO individuals to different populations because mtDNA haplotype matching to sources corresponded exactly. The weak assignment of one individual in SB to OD is more difficult to interpret.

Wildlife managers are sometimes confronted by newly discovered populations or stray individuals of unknown origin (e.g., Onorato et al. 2004, Latch et al. 2006*b*). Determining the origin may be critical to identifying the appropriate response. For instance, did the strays originate from a population of high conservation value? In California, where an Endangered Species Act (ESA)–listed subspecies (Sierra Nevada bighorn sheep [*O. c. sierrae*]) and an ESA-listed distinct population segment of desert bighorn sheep in the Peninsular Ranges occur in close proximity to unlisted populations of desert bighorn sheep, population genetic data may provide the best means for determining origin quickly. For instance, 2 small groups of bighorn sheep were documented in 2005 at the western edge of the Coso Range, where they have been absent for half a century (Wehausen 1999). Using DNA from fecal pellets collected in the vicinity of those sheep, microsatellites to distinguish individuals, and sequencing of mtDNA control region, 2 individuals with mtDNA haplotype E were identified (J. D. Wehausen, unpublished data). Haplotype E is common in the OD population (Fig. 1; Appendix A), which was the source of a reintroduction to the Argus Range immediately east of the Coso Range in 1986 (Bleich et al. 1990). Clearly, the newly detected individuals in the Coso Range were descendents of animals introduced into the Argus, rather than endangered Sierra Nevada bighorn. Similarly, Latch et al. (2006*b*) applied the mtDNA protocols we described here to determine that a newly detected population of bighorn sheep in Arizona originated from Rocky Mountain bighorn (*O. c. canadensis*) rather than desert bighorn populations.

Our findings demonstrate that translocations of bighorn sheep into habitat within 10–15 km of established populations may not always be necessary in the absence of other dispersal barriers. Translocation is expensive, sometimes unsuccessful, and comes at the biological cost of the individuals removed from the source population (Bleich et al. 1996). However, colonizations of CO and the Coso Range (above) suggest that translocated individuals may help maintain populations in nearby habitat patches and could help offset high population extinction rates.

Natural colonizations in this system have lower genetic diversity, like translocations described elsewhere (e.g., Hedrick et al. 2001, Whittaker et al. 2004). However, although A decreased in 3 of 4 and H_e declined in 1 of 4 colonizations that we examined (Table 2), genetic diversity still exceeded values reported in translocated populations of bighorn sheep in other locations (Gutierrez-Espeleta et al. 2000, Hedrick et al. 2001). For instance, using a different

set of 11 loci and samples sizes of 10–23 individuals/population, Whittaker et al. (2004) reported 2.2–2.4 alleles/locus and much lower H_e estimates (0.32–0.39) in translocated populations in Oregon but comparable estimates of 3.8 alleles/locus and $H_e = 0.57$ in one native population in Nevada, USA. Because natural colonization can result in continued interaction, genetic diversity may not decline as severely as after a translocation. For instance, we did not detect declines in H_e in the 2 cases where we observed radiocollared individuals traveling repeatedly between the source and colony (SB and IR). Thus, when human-made barriers threaten to block bighorn sheep dispersal (e.g., Flesch et al. 2010), translocation is less likely to be a successful strategy for mitigating loss of genetic diversity than maintaining natural connectivity.

MANAGEMENT IMPLICATIONS

Our data and data from Boyce et al. (1999) and recent extensive sampling in the northern desert from Death Valley to the Sierra Nevada (J. D. Wehausen, unpublished data) describe 42 unique mtDNA control region haplotypes among nearly all known bighorn sheep populations in California. These, coupled with microsatellite data, could be used to determine populations or regions of origin for future bighorn sheep colonizations. The colonizations we described and detected demonstrate that natural recolonization still helps maintain bighorn sheep across this region despite high rates of population extinctions (Epps et al. 2004). We recommend that populations described as extinct in previous surveys be resurveyed more frequently to determine whether recolonization has occurred. Populations reestablished by translocation served as sources for natural recolonizations of other nearby mountain ranges in 2 cases (SH and Argus), implying that translocation is an important tool for metapopulation management. However, because genetic diversity in colonizations did not decline as severely as has been reported for population translocations (e.g., Hedrick et al. 2001), we recommend maintaining connectivity and the potential for recolonization by avoiding disruption of natural dispersal routes and bridging anthropogenic barriers rather than relying solely on translocation. Known linkages between source populations and colonies should be protected.

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Appendix A. Mitochondrial DNA haplotypes (515 base pairs) from 394 bighorn sheep we sampled in 2000–2004 in 27 populations in southeastern California, USA. Haplotype 5 was first described by Boyce et al. (1999) in the Peninsular Ranges of California.

Code	Population	5	A	A2	B	C	D	E	F	G	H	I	J	K	M	N	O	P	Q	R	S	Total
CK	Clark					9									3							12
CL	Clipper		4			7			2					3								16
CO	Coxcomb						4		2			1										7
CU	Cushenbury															15						15
CV	Chemhuevi		7																			7
EABZ	Eagle-Buzzard Spring								18													18
EALP	Eagle-Lost Palms	3							10							1						14
GR	Granite		1			2	2	6	9			1										21
HA	Hackberry								13													13
IR	Iron				10	1																11
KD	Cady						4	1			5						2					12
LS	Little San Bernardino	1							9							2						12
MA	Marble					1			24	3												28
NE	Newberry								10	4												14
OD	Old Dad						7	12				6										25
OE	Indian Spring						1	10				1										12
OR	Orocopia								14									1	3			18
OW	Old Woman		3		18	5																26
PI	Piute Range					3					7									3		13
PR	Providence		8		3			2	6				1									20
QU	Queen								4							6			1			11
RG	Riverside Granite	3							5													8
SB	South Bristol								8	6												14
SG	San Gorgonio															17						17
SL	San Gabriel																				6	6
TU	Turtle		13	1																		14
WO	Wood					3			6		1											10
Total		7	36	1	31	31	18	31	140	13	13	9	1	3	3	41	2	1	4	3	6	394

Appendix B. Individual assignments of desert bighorn sheep sampled in 2000–2004 from 27 populations (rows) in California, USA, to genetic clusters (columns) using STRUCTURE (Pritchard et al. 2000). First number of each entry is number of assignments with assignment index >0.5 and the second number reflects total number of assignments to that cluster based on the highest assignment probability for each individual.

Code	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12	c13	c14
CK	1/1		10/11											
CL	8/8				8/8									
CO				4/5								2/2		
CU									15/15					
CV	7/7													
EABZ									0/1		10/12	3/4		
EALP											7/7	5/7		
GR	1/1	0/1		4/4			8/12		0/1				1/2	
HA			0/1			6/6	5/6							
IR								11/11						
KD				1/1	0/1								10/10	
LS											8/11	1/1		
MA	1/1	2/2			23/26									
NE		15/15												
OD				25/25										
OE				7/7									5/5	
OR										18/18				
OW			1/1					22/23				1/1		0/1
PI		0/2				9/10	0/1							
PR						2/4	16/16							
QU											6/6	4/5		
RG					0/2						2/2	4/6		
SB				0/1	12/13									
SG									17/17					
SL			6/6											
TU								1/1			1/1			10/12
WO						5/6	4/4							