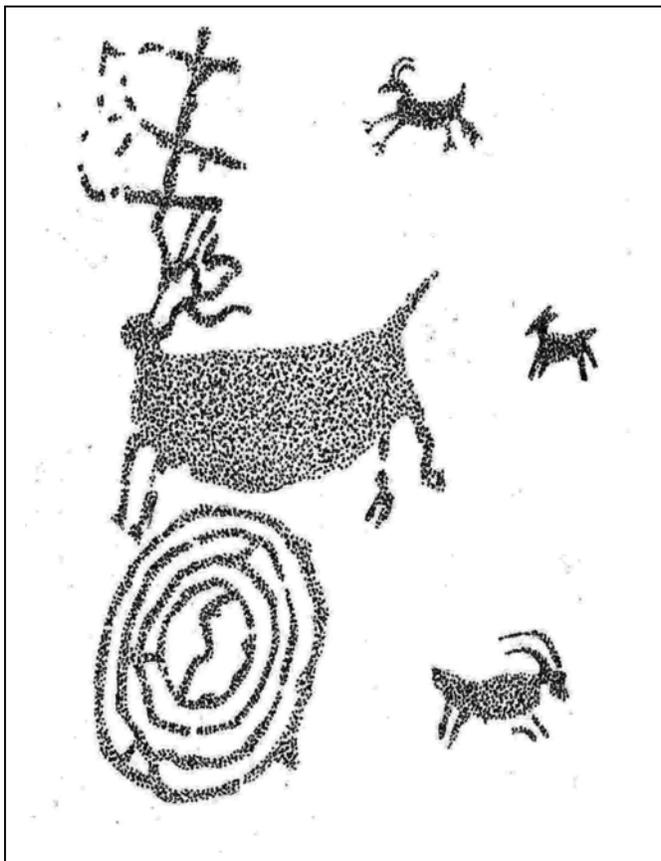


CHAPTER 4 USING GENETIC ANALYSES TO DESCRIBE AND INFER RECENT COLONIZATIONS BY DESERT BIGHORN SHEEP

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Abstract

Species occurring in small fragmented populations are often dependent on colonization or recolonization of empty habitat patches to persist, especially if local extinction is common. However, detecting natural colonizations is often difficult. Here we use genetic data, obtained primarily from fecal samples, to characterize recent colonizations of desert bighorn sheep (*Ovis canadensis nelsoni*) in southeastern California. We use inferences gained from known colonizations to determine the probable source population for another recent colonization. In another population we diagnose a probable cryptic extinction event, followed by recolonization from at least two source populations. We base these inferences on analyses of 14 microsatellite loci and 515 base-pairs of mitochondrial DNA control-region sequences, as well as the sex-identifying molecular marker SE47/SE48, obtained from 397

desert bighorn in 27 populations. We analyze colonization from the microsatellite data using conventional F-statistics, Bayesian population-level assignment tests, and individual-level assignment tests. We also map the distribution of mitochondrial DNA haplotypes to make inferences about the direction of gene flow between populations and to infer the movement of ewes between populations. All these types of data contained information that helped identify recent colonizations and source populations, particularly when used in combination. These genetic techniques provide powerful tools for monitoring systems of small, fragmented populations where direct census techniques are difficult or expensive to apply. We also present data describing the current genetic structure of desert bighorn sheep in southeastern California, to establish a base-line for future studies of population turnover.

Introduction

Managing species with metapopulation-like distributions requires understanding the processes of population extinction and population colonization. If the rate at which local populations in a fragmented system go extinct is not balanced by the recolonization rate of uninhabited patches, the system will decline to extinction across all patches (Levins 1969). However, developing accurate estimates of extinction and colonization rates requires particularly extensive data. Ideally, reliable repeated surveys have evaluated the presence or absence of the species of concern in all habitat patches, whether inhabited or not at the time of the survey (Hanski 1999). Because such data are rarely available, other tools are needed to provide insights into processes of population turnover. Here we explore the use of population genetic data to provide insights about recent colonization events in a natural metapopulation of desert bighorn sheep (*Ovis canadensis nelsoni*).

Population genetic analyses can be a powerful tool for identifying recent colonizations and determining the source of the colonizers, (e.g. Eldridge et al. 2001). However, the ability to distinguish between potential source populations depends on the structure of genetic variation within and between populations. Systems with large populations or many populations of recent common ancestry may provide little power to detect recent colonizations and identify source populations. Small populations, strongly affected by genetic drift, may show very strong spatial structuring of neutral genetic variation. Such rapid differentiation of populations improves the ability to detect recent colonizations. Because desert bighorn sheep populations in California are small (typically <50 individuals in size, Torres et al. 1994), genetic drift is strong and populations have rapidly developed strong spatial structure (Epps 2004).

Desert bighorn sheep are distributed through the desert mountain ranges of southeastern California. Because of their naturally-fragmented distribution, regional arrays of desert bighorn sheep populations appear to fit a metapopulation model (Schwartz et al. 1986, Bleich et al. 1990, Bleich et al. 1996). Frequent population extinctions have been documented (Wehausen 1999). Apparent colonizations also have been observed, but infrequently (Bleich et al. 1996). Here “colonization” refers to the permanent emigration of both sexes of desert bighorn sheep to an uninhabited patch of habitat, with subsequent reproduction. Detecting recent colonizations has proved difficult. Population surveys, conducted in California since the late 1930s (Wehausen 1999), have rarely completely examined areas thought to have few or no bighorn sheep. The time between surveys was often long. When sheep were detected in areas where population extinctions were thought to have previously occurred, it was not always clear whether the population truly had been extinct or just undetected. Populations also could have gone extinct and been recolonized between surveys, both events thereby undetectable. Even in cases where a

population appeared to be the result of a recent colonization, the source population was sometimes uncertain.

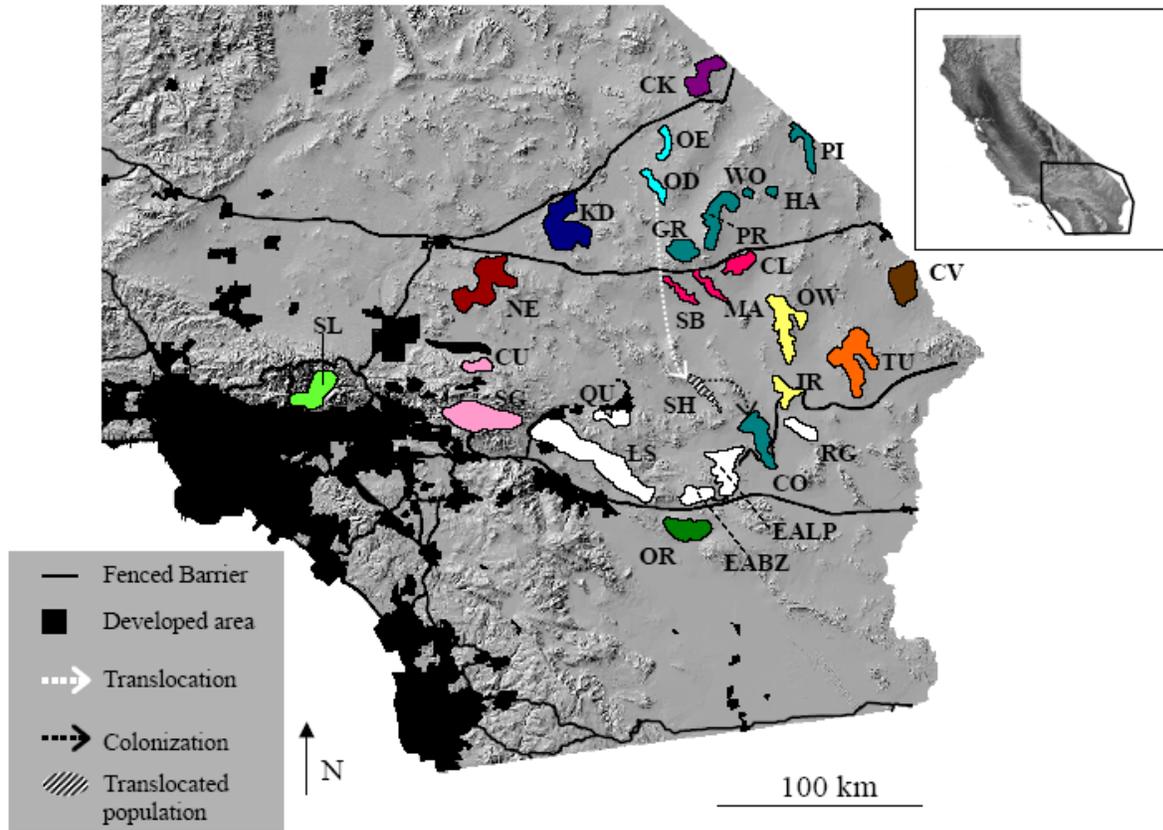


Figure 1. Relief map of southeastern California showing the 27 study populations sampled, as well as the reintroduced population in the Sheephole Mountains (“SH”). Population polygon coloring represents the results of BAPS clustering analysis; like-colored populations were clustered by genetic similarity, indicative of recent or current gene flow. Two-letter population identification codes are defined in Table 2. The colonization of the Coxcomb range (dashed black arrow) from the Sheephole Mountains was inferred from analyses in this paper.

Management of desert bighorn sheep in California could benefit greatly from accurate knowledge of where natural colonizations have occurred or may occur. Artificial emigrations (hereafter referred to as translocation) has been used by the California Department of Fish and Game (CDFG) to attempt reestablishment of viable desert bighorn populations in at least eight mountain ranges in California in the late 20th century (Bleich et al. 1996). Translocation is expensive, sometimes unsuccessful, and comes at the biological cost of the individuals removed from the source population (Bleich et al. 1996). Therefore, a clear understanding of the conditions under which natural recolonization is possible is needed. In some cases, the chances of natural recolonization may be so low that translocation is warranted. Identifying and understanding natural colonizations will help managers determine when translocation is necessary and when it is likely to be a waste of resources.

Using an extensive microsatellite and mitochondrial DNA sequence data set (details in methods), we examined two recent colonizations in the context of genetic variation throughout

the region. We used conclusions from these examples to infer the source population for a recently-discovered population of desert bighorn sheep and, in another mountain range, to detect evidence of another apparent recent colonization from multiple source populations. We used sex-identifying genetic markers to gain further insights on the source of females for this population. We also present the distribution of mitochondrial DNA (mtDNA) control-region haplotypes across southeastern California, to aid in the identification of future colonization events.

Study Populations

Radio telemetry and population surveys helped identify two recent colonization events in southeastern California in recent years. One event took place in the South Bristol Mountains (Figure 1, “SB”). No population of desert bighorn sheep was known to occur in the South Bristol Mountains at the time of the 1993 population inventory (Torres et al. 1994). However, three females radio-collared in the nearby (5 km) Marble Mountains (Figure 1, “MA”) were documented to travel to the South Bristol Mountains beginning in 1993 (documented by 2nd author). Initially only a single female was known to remain year-round in the South Bristol Mountains, where she bore a lamb (Bleich et al. 1996), but was subsequently joined by a second telemetered female and possibly other uncollared females that took up permanent residence there. By the late 1990s, a small but rapidly-increasing population had been established.

The second apparent colonization event occurred in the Iron Mountains (“IR”, Figure 1) with the Old Woman Mountains (“OW”, Figure 1) as the apparent source population. An inhabitant of the former town of Milligan, in the region between these two mountain ranges, reported regularly seeing tracks from sheep crossing between the Old Woman and Iron Mountains in the early 20th century (observation recorded by 2nd author). A telemetered female from the Old Woman Mountains crossed to the Iron Mountains to have her lamb in the mid 1980s, but no evidence of other sheep there was found at the time (observed by 2nd author). Observations at the Lutz big-game guzzler in the Iron Mountains indicated no resident sheep at the time of the 1993 population inventory. However, extensive sheep sign was observed in 2000 (G. Sudmeier, personal communication) and males, females, and juveniles were apparently to be resident in this mountain range during field work from 2001-2003 (observed by the 1st author). Bighorn were subsequently radio-collared in the Iron Mountains and nearby Old Woman Mountains and collared males documented to move between those ranges on several occasions since 2001 (A. Pauli, CDFG, personal communication). We use “colonization pair” to describe two populations linked, as in these examples, by a recent colonization event.

After describing those known colonizations, we used the analyses in this paper to infer the source of the Cushenbury population (Figure 1, “CU”). This small, isolated population was discovered in the 1980s and is thought to be the result of a recent colonization (J. Davis, personal communication). Populations in the Newberry Mountains population (Figure 1, “NE”) and San Geronio Peak (Figure 1, “SG”) both have been considered as possible sources.

Materials and Methods

We used genetic data set for 397 individuals from 27 populations of desert bighorn sheep in southeastern California (Figure 1), developed from fecal and blood samples collected during 2000-2004. Each individual was genotyped at 14 microsatellite loci, and 515 base pairs of mitochondrial DNA control region were sequenced for 394 of these samples. Mean sample size per population was 15 individuals; the range of sample sizes per population was 6-29 with a standard deviation of 5.9. Complete details of sample collection, genetic analyses, individual

genetic identification to remove duplicate fecal samples, and analyses of error are described in Epps (2004). Fecal samples were collected primarily in summer when desert bighorn make frequent use of surface water for drinking (Turner and Weaver 1980); we collected samples at all known major water sources in each population in an attempt to obtain a representative sample of all individuals in the range.

Microsatellite loci are highly variable nuclear DNA markers; individuals inherit microsatellite alleles from both parents. Given enough loci (variable regions analyzed), microsatellite markers can be used to detect demographic events in populations such as recent gene flow and bottlenecks. We based our initial inferences about recent colonizations on these data. Mitochondrial DNA is inherited from the mother via the egg, and its sequences (haplotypes) typically show less variation within a population. We used mtDNA variation to help determine the source of the females in each colonization event. Because female bighorn are generally more philopatric than males (Geist 1971), female dispersal limits colonization: thus, it was important to have a means of distinguishing male- and female-mediated dispersal.

We determined the sex of each individual sampled using the SE47/SE48 sex identification primers (Yamamoto et al. 2002). SE47/SE48 primers amplify one DNA fragment for females and two DNA fragments for males. Failed reactions can thus be distinguished by lack of any amplification products. However, because “allelic dropout” is a common problem when using degraded DNA samples, we replicated amplifications at least three times for female samples. In the event of a male band being detected, no further amplifications were conducted. We used 20 μ L PCR reactions with the following reaction conditions: 1x PCR Buffer I (Applied BioSystems), 0.16 mM dNTPs, 10 μ g bovine serum albumin (New England BioLabs), 1.9 mM MgCl₂, 400 nm each primer, and 0.6 units of Amplitaq Gold™ DNA polymerase (Applied BioSystems). We used an initial heating cycle of 95° C for 7 minutes 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/SE48 amplification products on 2% agarose gels, pre-stained with ethidium bromide.

We used a series of techniques to describe the genetic structure of the known recent colonizations. We first used GENEPOP (Raymond and Rousset 1995) to calculate F_{ST} (Wright 1921, Weir and Cockerham 1984) between all population pairs from the microsatellite data described above. F_{ST} is a measure of shared genetic variation between two groups; an F_{ST} of 0 implies complete mixing, while an F_{ST} of 1 implies no shared genetic variation. While F_{ST} is sometimes difficult to interpret because it is influenced by past and present gene flow, it still provides a useful relative estimate of population similarity (Neigel 2002). We compared F_{ST} values between each population of interest and nearby populations and inferred the most likely source population as that with the lowest F_{ST} value.

We next used the program BAPS (Corander et al. 2003) to investigate whether population-level assignment tests could detect known recent colonization pairs, and then to infer the source of the Cushenbury population. BAPS uses Bayesian Markov-chain Monte Carlo simulations to assign populations to clusters based on the genetic similarity of the individuals within. Eldridge et al. (2001) found Bayesian clustering methods to perform the best of the available assignment tests when attempting to determine source populations for recent colonizations. We set burn-in time to 10,000, chain length to 50,000, thinning to 5, and checked to insure that these values were sufficient to achieve convergence of estimates (Corander et al. 2003). BAPS provides posterior probabilities for various levels of population clustering; we used only the clusters defined to have posterior probability of >95%.

Table 1. F_{ST} values for all sampled populations, estimated using GENEPOP from 14 microsatellite loci. All values were significantly different. Known recent colonization pairs have solid outlines, inferred recent colonization pair (SG-CU) has dotted outline. Two-letter population codes are defined in Table 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

We mapped the distribution of mtDNA haplotypes across the sampled populations to evaluate the directionality of colonization in recent colonization pairs. We predicted that known recently-colonized populations would contain a sub-set of the haplotypes present in the source populations, since presumably only a small number of females actually travel to the newly-colonized area.

After detecting another likely recent colonization, we used individual-level assignment tests to explore whether this population was derived from multiple source populations. We combined all individuals from all populations into a single data set and used the program STRUCTURE (Pritchard et al. 2000) to estimate the number of “populations” (clusters of individuals apparent from their genetic similarity) in the sample. STRUCTURE then fractionally assigns each individual to each cluster. We determined the most likely cluster number using a burn-in of 10,000 chains followed by 100,000 chains for each putative number of clusters, limiting the values tested to those between 10 and 30. The most likely cluster number had the negative log-likelihood value closest to zero. Using the most likely cluster number, we examined the composition of each cluster and recorded which individuals from which populations were grouped together.

Results

F_{ST} values for the known recent colonizations were less than 0.05, indicative of high levels of gene flow between these population pairs (Table 1). For the South Bristol colonization, the F_{ST} value with the Marble Mountains was lower than that for any other population (Table 1). The F_{ST} value between the Old Woman Mountains and the Iron Mountains was likewise much lower than other potential source populations for the Iron Mountains colonization, such as the Riverside Granite Mountains (Figure 1, “RG”; $F_{ST} = 0.17$) or the Coxcomb Mountains (Figure 1, “CO”; $F_{ST} = 0.16$) (Table 1). The F_{ST} value between the Cushenbury population and the Newberry Mountains population ($F_{ST} = 0.37$) was much higher than that between the Cushenbury and San Gorgonio populations ($F_{ST} = 0.07$) (Table 1).

Population clusters created by Bayesian assignment clustered the Marble Mountains with the South Bristol Mountains and the Old Woman Mountains with the Iron Mountains (Figure 1). The Cushenbury population clustered with San Gorgonio Peak rather than the Newberry Mountains. BAPS population clusters also reflected the isolating effects of distance (e.g. Newberry, San Gabriel, and Chemhuevi populations; Figure 1, “NE”, “SG”, “CV”).

The Coxcomb Mountains population clustered with the Providence, Hackberry, Wood, and Piute population cluster, over 95 km to the north (Figure 1, “PR”, “HA”, “WO”, “PI”). This initially counter-intuitive result appears to have resulted from a cryptic colonization event: the Coxcomb Mountains are in close proximity to the Sheephole Mountain population (Figure 1, “SH”). The Sheephole Mountains population, extinct or nearly extinct, was reestablished by translocation of desert bighorn sheep from the Old Dad Peak population in 1984 and 1985 (Bleich et al. 1996). The Coxcomb Mountains population now appears to consist largely of sheep with alleles matching those present in the populations north of I-40, apparently due to colonizing sheep from the Sheephole Mountains. The F_{ST} value between the Coxcomb Mountains and Old Dad Peak was only 0.06, lower than any other F_{ST} values for comparisons involving the Coxcomb Mountains (Table 1). However, BAPS analysis grouped the Coxcomb Mountains with the Providence Mountains population cluster (Figure 1, “PR”, “WO”, “HA”, “PI”), rather than the Old Dad/Indian Spring cluster. This may have been the result of multiple source populations for the Coxcomb Mountains sheep: an admixture of Old Dad Peak bighorn from the Sheephole

Mountains and bighorn from nearby local populations might have changed allele frequencies in the Coxcomb Mountains population enough to cause this confusing result.

We detected 19 mtDNA haplotypes in these 27 populations (Table 2). Haplotype sequences are recorded in GenBank. Only one of these haplotypes, haplotype “5B” or haplotype “5” by Boyce’s nomenclature, was formerly described by Boyce et al. (1999) in the “Peninsular” ranges to the southwest of this study area. The South Bristol Mountains contained two haplotypes, “F” and “G”, while the Marble Mountains contained haplotypes “F”, “G”, and “C” (Table 2). The Iron Mountains contained two haplotypes, “B” and “C”, while the Old Woman Mountains contained haplotypes “A”, “B”, and “C” (Table 2). The Cushenbury population contained only one haplotype “N”, also found in the San Gorgonio population, and found elsewhere only in the Queen (Figure 1, “QU”), Little San Bernardino (Figure 1, “LS”) and the Eagle Mountains-Lost Palm (Figure 1, “EALP”) populations (Table 2). None of the Newberry Mountains haplotypes (“F” and “G”) were discovered in the Cushenbury population. Five bighorn sampled in the Coxcomb Mountains had haplotypes “D” or “I”, found elsewhere only at Old Dad Peak and associated populations north of Interstate 40. The remaining two bighorn had haplotype “F”, which is ubiquitous in the nearby Eagle Mountains-Buzzard Spring (Figure 1, “EABZ”) population. These findings gave further support to the hypothesis of multiple source populations for the Coxcomb Mountains population.

STRUCTURE detected 18 population clusters from the microsatellite data. We examined individual assignments for the Coxcomb Mountains population to further test the hypothesis of multiple source populations. Using the SE47/48 data, we determined the sex of the individuals in question as well. Four males and one female were assigned to the same cluster as the bighorn sheep sampled at Old Dad Peak; these five sheep also had Old Dad Peak-type mtDNA haplotypes “D” or “I”. The remaining two males were assigned to the same cluster as many of the bighorn sheep from the Eagle Mountains-Buzzard Spring population; these individuals had the mtDNA haplotypes “F” so commonly found in Buzzard Spring (and unknown at Old Dad Peak).

Discussion

The genetic analyses described here successfully detected known recent colonizations, allowed a strongly-supported inference of the source population for another recently-colonized population, and detected a cryptic colonization in the Coxcomb Mountain population. This cryptic colonization appears to have resulted from multiple source populations, based on the mtDNA haplotypes and the findings of the individual assignment tests. No females with “local” haplotypes were detected in this tiny population, suggesting that the Coxcomb Mountains may have experienced an extinction followed by a recolonization by females from the Sheephole Mountains, rather than merely augmentation by the Sheephole Mountains bighorn sheep. Because the individuals assigned to Old Dad Peak also had Old Dad mtDNA haplotypes, the colonization may be very recent: at this time little interbreeding appears to have occurred between the Sheephole (Old Dad) bighorn and Eagle Mountain bighorn present in the Coxcomb Mountains. As the population becomes more mixed, some bighorn would likely be assigned by microsatellite data to a different source population than that indicated by their mtDNA haplotypes.

Table 2. Mitochondrial DNA haplotype (515 base pairs) distribution from 394 bighorn sheep sampled across 27 populations. Haplotypes are arbitrarily assigned letters; haplotype “5B” is Boyce et al.’s (1999) haplotype “5”, first described in the Peninsular Ranges of California.

Code	Population	5B	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
EAB	Eagle-Buzzard																					18
Z	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	Orocopeia									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

F_{ST} values proved useful for evaluating specific hypotheses about the identity of source populations when evaluating a particular recent colonization. For both known recent colonizations, F_{ST} values with the known source population were lower than for other nearby populations. F_{ST} comparisons also supported the findings of the other methods presented here that San Gorgonio Peak population was the source of the Cushenbury population, and that the Coxcomb Mountain population was derived originally from Old Dad Peak. However, because there is no good means to assign statistical confidence to the relative differences in F_{ST} values, this technique has limited value. Simulated data sets based on the effective population sizes and migration rates characteristic of a given study system might provide useful guidelines when using this method: different scenarios could be evaluated to see if observed differences in F_{ST} values are meaningful. F_{ST} values do not easily allow the detection of multiple source

populations, particularly if only a few individuals are present from the additional source population(s) as was the case in the Coxcomb Mountains.

Population clustering using BAPS detected both the known recent colonizations. BAPS also grouped the Cushenbury population with the San Gorgonio population, and detected an ambiguity in genetic structure that led to the description of a “cryptic” colonization of the Coxcomb Mountains from the Sheephole Mountains. Such population-level clustering analyses appear to be an excellent descriptive technique for identifying regions of recent or current gene flow. However, despite the ability to estimate the posterior probability of these clusters, it is unclear how much time without gene flow is necessary to cause populations to be grouped separately. The length of this time period would be determined by the typical effective population sizes of the populations concerned, and also would be affected by population bottlenecks or founder events. For the small, rapidly-fluctuating populations of desert bighorn sheep in California, even a few decades of separation appear to be sufficient to affect population clustering. For instance, the Marble Mountains were clustered separately from the Granite (Figure 1, “GR”) and Providence Mountains (Figure 1, “PR”), despite a geographic distance between the Marble and Granite Mountains of <5 km (Figure 1). This genetic separation is apparently the result of the disruption of gene flow by Interstate 40, constructed about 40 years ago (Epps 2004). In systems with larger, more stable populations, BAPS population-level clustering may not have the ability to detect such recent events. Again, simulating the observed genetic divergence, using effective population sizes and migration rates estimated from the data, might provide rough guidelines for estimating the time of separation and thus the resolution provided by these clustering algorithms.

Mapping the occurrence of mtDNA haplotypes was useful because it provided a female-only map of dispersal patterns to compare with the male- and female-mediated dispersal patterns reflected by the microsatellite data. Because female movements are the limiting factor for successful colonization, this information could be quite helpful for management purposes. For instance, no mtDNA haplotypes from the Newberry Mountains were detected in the Cushenbury population, supporting a southern origin for the females in this population. The mtDNA information helped determine that the Coxcomb Mountains population was at least partially founded by females from the Sheephole Mountains, rather than merely attracting males from that location. Mitochondrial DNA also provided some inferences about the direction of gene flow: in both previously-known recent colonization pairs, the colonized population had only a subset of the haplotypes found in the source population. Thus, even if managers can only analyze mtDNA haplotypes due to lack of funding or appropriate markers, this technique alone can still provide a useful management tool. Mitochondrial DNA is easy to extract from mammalian cells and relatively cheap to analyze compared to microsatellite markers. Sex-determining markers such as SE47/SE48 also proved useful for describing the Coxcomb Mountains colonization, and in general could be useful when determining population origins for species with sex-biased dispersal. In the case of desert bighorn sheep, these markers could also help determine whether a mountain range supports both females and males or merely serves as temporary habitat for males.

Using individual-based assignment tests, particularly in combination with sex-identification data in the case of non-invasive genetic samples, also can provide useful insights as evidenced here. However, the use of these tests to identify the multiple source populations for the Coxcomb Mountains was particularly effective because one potential source, the Sheephole Mountains, originated in a very distant population rather than other, similar nearby populations. In cases

where all populations are of local origins, clear conclusions may be more difficult.

Efforts to identify the source population for a recent colonization will always be hampered the presence of several nearby populations with similar genetic structure due to recent common origins. For this reason, it is important to genetically characterize all the known populations in a region to be able to make any inferences. There can be no absolute F_{ST} value, for instance, that implies a recent colonization in all systems. Using genetic analyses to track recent gene flow will require careful examination of the degree and geographical scale of genetic structure, and will probably be most effective in systems with small population sizes and strong isolation by distance. All the methods described here are useful primarily as tools for inference rather than “tests”; enough data must be obtained to allow such comparative techniques to be used with some confidence.

Genetic tools, such as those described here, can provide powerful tools for monitoring and making management decisions about systems of fragmented populations. A great deal of information can be obtained from non-invasive samples such as fecal pellets. In future management of desert bighorn sheep in California, as populations continue to go extinct and be recolonized, a relatively small amount of additional genetic sampling could provide important comparisons to the baseline data that we have established here.

Finally, the findings of this paper reflect a successful case of “metapopulation management” for desert bighorn. Here, a population reestablished by translocation served as the source of a “natural” recolonization of an adjacent mountain range that apparently suffered near or complete population extinction. Because of the strong variation in climate conditions from year to year, and a general trend of increasing aridity, local extinctions of bighorn populations in the California desert have been common and will continue to be so (Epps et al. 2004). Translocation remains a valuable tool for “buffering” this system of small fragmented populations. Maintaining natural dispersal routes, for instance by limiting construction of major highways through key dispersal routes, is also important to maintain viable populations of desert bighorn sheep in the California deserts. Natural recolonization still plays a critical role in maintaining bighorn sheep across this region.

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