Articles

Desert Bighorn Sheep: Changes in Genetic Variation Over Time and the Impact of Merging Populations

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Abstract

Founder effects, genetic bottlenecks, and genetic drift in general can lead to low levels of genetic diversity, which can influence the persistence of populations. We examine genetic variation in two populations of desert bighorn sheep *Ovis canadensis* from New Mexico and Mexico to measure change over time and evaluate the impact of introducing individuals from one population into the other. Over about three generations, the amount of genetic variation in the New Mexico population increased. In contrast, over about two generations the amount of genetic variation in the Mexican population decreased by a great extent compared with an estimate from another Mexican population from which it is primarily descended. The potential reasons for these changes are discussed. In addition, although both populations have low genetic variation, introduction of Mexican rams into the New Mexico population might increase the amount of genetic variation in the New Mexico population. Overall, it appears that management to increase genetic variation might require substantial detailed monitoring and evaluation of ancestry from the different sources and fitness components.

Keywords: effective population size; genetic drift; genetic rescue; heterozygosity

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Introduction

A fundamental challenge of conservation biology is to understand the factors that influence the size and persistence of small populations. Demographically, persistence of small populations can be influenced by a number of environmental factors such as weather, food availability, competitors, predators, pathogens, and human harvesting. To avoid extinction and preserve evolutionary potential, the population number necessary for long-term viability of isolated small populations might be substantial (Traill et al. 2010).

Genetic factors such as genetic drift (chance genetic changes due to small population size) and inbreeding (mating between relatives) are also potentially important factors influencing extinction probability and maintenance of evolutionary potential (Frankham et al. 2010; Allendorf et al. 2013). More specifically, an effective population size (the population size that assumes that each parent has an equal chance of producing a given offspring) of 50 has been suggested to avoid inbreeding depression and an effective population size of 500 has been suggested to maintain genetic variation for future adaptation (see discussion in Jamieson and Allendorf 2012). Genetic variation within a small population can be greatly enhanced by natural gene flow from other populations in functional metapopulations (a larger population composed of subpopulations that are connected by migration). To overcome the loss of genetic variation and the consequent lowered fitness in small

Table 1. A summary of the information discussed for the three populations, Red Rock, Tiburon, and Pilares (sample year, sample size, and table in text where data are presented) and the data source, Gutiérrez-Espeleta et al. (2000), Hedrick et al. (2001), and this study (Hedrick and Wehausen 2014).

Population	Sampling year	Sample size	Data for 8 shared loci	Data for 10 additional loci	Data source
Red Rock	1993	25	Table 2	—	Gutiérrez et al.
Red Rock	2009	27	Table 2	Table 4	This study
Tiburon	1998	14	Table 3	—	Hedrick et al.
Pilares	2011	10	Table 3	Table 4	This study

isolated populations, either natural gene flow or human-mediated gene flow can produce genetic rescue (an increase in fitness following gene flow or translocation indicating lower fitness of a population before gene flow; Tallmon et al. 2004; Hedrick and Fredrickson 2010).

Bighorn sheep *Ovis canadensis* disappeared from much of their native habitat beginning in the mid-19th century, coincident spatially and temporally with the introduction of domestic sheep (Wehausen et al. 2011) and unregulated hunting. In the United States, only 15,000–18,200 bighorn sheep remained by 1960, of which the number of desert bighorn sheep in the southwestern United States numbered only about 7,700–8,100 (Buechner 1960). Subsequent attempts to reverse these trends involved limitation on hunting, protection of some populations, removal of domestic sheep, major restoration efforts via translocation, and water developments for desert bighorn sheep. Bighorn sheep populations are a potential indicator of the health of many mountainous environments in western North America and their conservation has been strongly advocated by conservation organizations, hunting groups, and state and federal agencies.

The relatively small size of many desert bighorn sheep populations has long been apparent (Trefethen 1975) and would predict a strong influence of genetic drift and inbreeding. It is also well-recognized that most bighorn sheep populations exist within a metapopulation structure (Schwartz et al. 1986; Bleich et al. 1996). Resulting gene flow between subpopulations counteracts the negative effect that small population size would otherwise have on reducing genetic diversity (Epps et al. 2006). However, this is not the case for bighorn sheep populations that are genetically isolated in the wild or in captivity. Those situations, and the major past perturbations that many bighorn sheep populations and metapopulations have experienced, provide good reasons to raise and research genetic questions that relate to long-term population viability.

Population genetic information concerning bighorn sheep has accumulated significantly in recent years. Several populations of bighorn sheep that are small or isolated, have experienced significant bottlenecks (temporary small population sizes that might cause genetic drift), or are descended from relatively few founders have much lower genetic variation than other populations (Gutiérrez-Espeleta et al. 2000; Ramey et al. 2000; Hedrick et al. 2001; Whittaker et al. 2004; Hogg et al. 2006; Johnson et al. 2011). The impact of inbreeding on fitness-related traits has been found in captive bighorn sheep (Sausman 1984; but see Kalinowski and Hedrick 2001) and in wild populations (Hogg et al. 2006; Johnson et al. 2011; Rioux-Paquette et al. 2011). In addition, significant genetic rescue was documented in one wild population (Hogg et al. 2006; Miller et al. 2012). Overall, where sufficiently detailed examination and statistical power exist, evidence for the negative impact of low genetic variation from genetic drift and inbreeding in bighorn sheep populations has generally been found.

Here we examine genetic variation in detail and the potential impact on fitness in two important populations of desert bighorn sheep from Red Rock, New Mexico, USA, and Tiburon, Sonora, Mexico, and its descendant population in Pilares, Coahuila, Mexico. The Red Rock population has been the source for translocations into multiple populations in New Mexico and the Tiburon population has been the source for translocations into multiple populations across northern Mexico. The Pilares population has also been used recently to introduce new genetic variation into the Red Rock population.

The examination of genetic variation over multiple generations in the population from Red Rock and Tiburon and its descendant population Pilares provides a framework for evaluating the potential impact of genetic drift and inbreeding in these two important source populations. In addition, the genetic examination of the Pilares and Red Rock populations presents the background for understanding the potential success of translocations and genetic rescue in bighorn sheep populations. These analyses should contribute both in general to comprehending the genetic impact of small populations on extinction and the significance of translocation in endangered species and to the management of desert bighorn sheep, an important and recovering species.

Methods

Populations

For the convenience of the reader, a summary of the genetic information for the different populations, their sample year, their sample size, and table in text where data are presented and the data source is given in Table 1.

Red Rock. The Red Rock Wildlife Area is a 1,530 acre (619-ha) fenced captive breeding facility for desert bighorn sheep in southwestern New Mexico (Figure 1). Desert bighorn sheep in the state of New Mexico reached a low number of fewer than 70 in 1980. The Red Rock population was established in 1972 from five

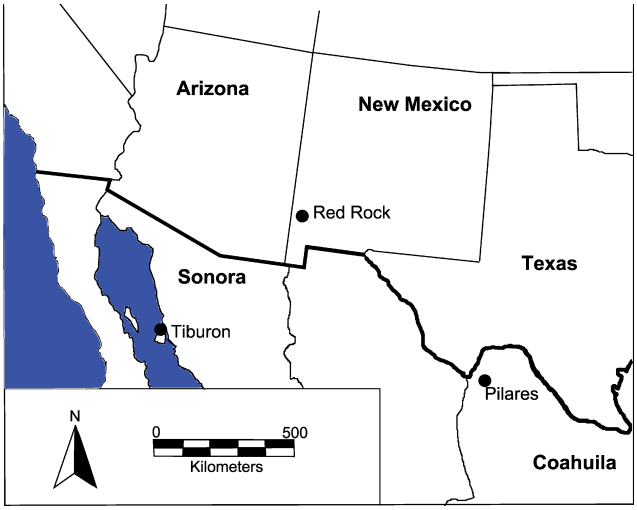

Figure 1. Map of the adjoining area in the United States and Mexico where the three populations of desert bighorn sheep *Ovis canadensis*, Tiburon Island (sampled in 1998), Red Rock (sampled in 1993 and 2009), and Pilares (sampled in 2011) are located.

ewes from the area near Pico Johnson, Sonora (W. Montoya, New Mexico Department of Game and Fish, personal communication,) and eight sheep (three rams and five ewes) from the native population in the San Andres Mountains, New Mexico (about 120 km east of Red Rock). In 1975, eight more ewes from the San Andres Mountains were added to the population; thus, the population was started with 3 rams and 18 ewes from two different sources (E. Rominger, New Mexico Department of Game and Fish, personal communication; Hedrick 2013). However, four of the original Mexican ewes were pregnant by wild Mexican rams when they were brought into the facility and four of the 1975 ewes from the San Andres Mountains were pregnant by wild San Andres rams. Assuming that eight different rams sired these eight lambs, the population might have been started by as many as 11 rams and 18 ewes. Using these data and the potential probability of parentage from the rams and ewes present in different years, Hedrick (2013) estimated that the effective number of Red Rock founders was 16.7.

The Red Rock population grew to 144 in 1995 and since 1979 has been the source of >400 bighorn sheep translocated to various areas in New Mexico in an effort to increase sheep distribution and numbers of wild desert bighorn sheep. Since 1975 there were no planned introductions into this population until 2011, when 10 more rams from the captive population from Pilares, Coahuila, Mexico (Figure 1), were added to the Red Rock population. However, three rams appeared outside the enclosure in 2007 and were eventually let in after being determined genetically to be desert bighorn sheep. At the time they were thought to be returning escapees, but later data indicate otherwise (see below).

Tiburon Island. There is no recent history of native bighorn sheep on Tiburon Island (Figure 1) in the Sea of Cortez, but the habitat there is similar to that on nearby mainland Sonora, where there are native bighorn sheep. In early 1975, 20 desert bighorn sheep (4 males and 16 females) were captured near Punta Chueca, Sonora, and translocated to Tiburon Island (Montoya and Gates 1975). There is no detailed information on the contributions of these initial individuals to the population, or detailed data on the population size over time, but the population increased very quickly, apparently because of good forage, ample water, and lack of predators and livestock on the island.

Hedrick (2013) assumed that the number of effective males in the founding group was one or two, and estimated the number of effective Tiburon founders at 3.8 or 6.4, respectively. In an aerial survey, 293 sheep were observed on three mountain ranges on Tiburon Island (Sierra Kun Kaak, Sierra Tiburon, and Sierra Menor) in 1993 (Lee and Lopez-Saavedra 1994). Using the Hervert et al. (1998) sightability estimate and assuming that only 75% of the sheep habitat was surveyed (R. Lee, Arizona Game and Fish Department, personal communication), a total population estimate of 848 bighorn sheep on Tiburon results. As a result of the fast growth of the population, there has been extensive hunting of bighorn sheep on the island, and since 1996 >500 sheep have been translocated from this population to other areas in northern Mexico to establish other populations (Wakeling et al. 2009).

Pilares. In 2000, the Mexican company CEMEX (one of the largest suppliers of building materials and cement in the world) built a 4,900-ha bighorn breeding facility in the Chihuahuan Desert within a 32-km perimeter fence that includes Sierra Pilares. This facility was originally stocked with 48 bighorn sheep from four herds. Three of these herds were descended from Tiburon Island animals, while the fourth was from nearby Sierra Punta Cirios on the Sonoran mainland. In 2010, this herd was estimated at 300 and animals have been translocated from there to New Mexico, Texas, and other sites in northern Mexico. In 2011, 10 rams from Pilares, Mexico, were introduced into the Red Rock population with the purpose of increasing genetic variation.

Genetic analyses

Gutiérrez-Espeleta et al. (2000) estimated genetic variation in populations of desert bighorn sheep using 10 dinucleotide microsatellite loci, including 25 sheep from Red Rock sampled between 1992 and 1994 (here for simplicity we will use the average of these two times, 1993). They found that the Red Rock population had the lowest heterozygosity (0.357) and average number of alleles (2.40) of the 13 populations examined. Hedrick et al. (2001) used the same 10 microsatellite loci to examine genetic variation in 14 sheep from Tiburon Island in 1998 and found slightly higher heterozygosity (0.412) and average number of alleles (2.50) and ranked it second lowest, after the Red Rock population, for the populations sampled.

Here we present genetic data for 18 dinucleotide microsatellite loci (Tables S1, S2, *Supplemental Material*) using blood samples from 28 rams removed in 2009 from Red Rock and the 10 rams from Pilares that were

introduced to the Red Rock population, of which 8 loci also were used by Gutiérrez-Espeleta et al. (2000) and Hedrick et al. (2001).

All amplifications were 14-uL reactions that included 3.64 uL of 1:30 DNA dilutions with an extra 0.7 uL of H_2O to counteract initial dry-down run in 96 well plates that included one positive and one negative control and two independent amplifications for each DNA sample. We amplified 15 loci in two multiplex polymerase chain reactions (PCRs; FCB266, MAF209, FCB304, JMP29, FCB11, AE129, BL4; and AE16, TCRBV62, MMP9, MAF48, MAF33, FCB193, MAF65, OMHC1). Each of those reactions received 7 uL of Qiagen Multiplex PCR Mix, primer concentrations varying from 0.107 to 0.35 uM, and H_2O to make final volume. Thermocycling for both was 15 min at 95°C; 40 cycles of 30 s at 94°C, 90 s at 60°C, 60 s at 72°C; and a final 30 min at 60°C. The loci MAF 36 and HH62 were multiplexed in a separate PCR with 3 mM $MgCl_2$, 0.179 uM (MAF 36) and 0.207 uM (HH62) primer, while TGLA387 was amplified alone with 3.375 mM $MgCl_2$ and 0.429 uM primer; PCR conditions common to these reactions were 1 × PCR buffer (Applied Biosystems), 0.4 ug/uL BSA (New England Biolabs), 160 uM dNTP, and 0.035 units/uL Amplitaq Gold (Applied Biosystems). Thermocycling was 7.5 min at 93°C followed by 40 cycles of 95°C for 30 s, 56°C (MAF36/HH62) for 40 s or 53°C (TGLA387) for 50 s, and 72°C for 30 s, with a final step of 5 min at 60°C. The PCR products (0.8 uL for Multiplex Mix amplifications; 0.9 uL for MAF36/HH62; and 1.2 uL for TGLA387) were dried down and then rehydrated with 1.5 uL deionized formamide, 0.4 uL Tamra 350 size standards (Applied Biosystems), and 0.2 uL Blue Juice (Invitrogen), and run on an ABI 377 sequencer in 96-lane mode with different loci in adjacent lanes.

We scored chromatograms manually using GeneScan 3.1.2 software (Applied Biosystems). For each locus, we tested conformance with Hardy–Weinberg expectations in Genepop software (Rousset 2008) using the exact or Markov Chain test with default parameters.

Theory

Below is the formula that we used to calculate the expected heterozygosity over time, given a specific effective population size and that other factors such as selection, mutation, inbreeding, and gene flow are not as important (Hedrick 2011). The heterozygosity expected from genetic drift after t generations, given that the initial heterozygosity is H_0, is

$$H_t = H_0 \prod_{i=1}^{t} \left[1 - \frac{1}{2N_{e.i}}\right] \quad (1)$$

where $N_{e.i}$ is the effective population size in the ith generation (Hedrick 2011). For the comparison of Tiburon and Pilares samples, we assumed two generations between them (12 y between 1998 and 2010; for the Red Rock sample, the heterozygosity actually increased so that this approach is not appropriate). The effective population sizes for the first two generations for Tiburon–Pilares (the population originated from Tiburon Island and whose descendants are now at Pilares) was assumed to be equal and in the last generation the size of the sample was 10 for Pilares, because at this point there had been no differential reproduction, etc., which would make the population size and the sample size different.

To determine the probability that the heterozygosity observed in the Pilares sample would occur, given the initial observed allele frequencies in the Tiburon sample and a specific effective population size, we used a genetic simulation program written by the first author. For each N_e value examined, we generated 10,000 independent samples by simulation initiated with the observed allele frequencies in the Tiburon sample. We calculated both the average heterozygosity over samples and the proportion of samples that had $H < 0.322$ (the uncorrected observed heterozygosity in the Pilares sample).

We calculated the heterozygosity in a population that is composed of two source populations (in this case Red Rock and Pilares) in the following ways. The average expected heterozygosity for a given locus in a population before any breeding that is composed of a proportion RR sheep from Red Rock and a proportion PI sheep from Pilares ($RR + PI = 1$), each in Hardy–Weinberg proportions, is

$$H_E = RR\left(1 - \sum p_{i.RR}^2\right) + PI\left(1 - \sum p_{i.PI}^2\right) \quad (2)$$

where $p_{i.RR}$ and $p_{i.PI}$ are the frequencies of the ith allele in the Red Rock and Pilares populations.

The observed heterozygosity in the first-generation progeny, assuming in this case that all Pilares sheep are males and that a proportion PI' of the males are from Pilares and a proportion RR' are males from Red Rock is

$$H_O = PI' \sum_{i \neq j, i<j} p_{i.RR} p_{j.PI} + RR'\left(1 - \sum p_{i.RR}^2\right) \quad (3)$$

Here it is assumed that progeny that are the result of matings between Red Rock rams and ewes are in Hardy–Weinberg proportions.

In the second generation, assuming Hardy–Weinberg proportions in all the progeny, the expected heterozygosity is

$$H'_E = 1 - \sum \bar{p}_i^2 \quad (4)$$

where $\bar{p}_i = (0.5PI' + RR')p_{i.RR} + 0.5PI'p_{i.PI}$ and is the average frequency of the ith allele determined by the expected proportions of ancestry from Red Rock and Pilares.

We calculated the genetic distance using standard genetic distance of Nei (1987), and we calculated the expected heterozygosity using the small sample size correction (Nei 1987).

Results

It is widely recognized that the measured sizes of microsatellite alleles vary as a function of the device used for measurement, the particular run on that device, the PCR conditions used, and even the fluorescent dye label

Table 2. The frequencies of alleles and heterozygosity (number of alleles) at eight microsatellite loci in the two studies of desert bighorn sheep *Ovis canadensis* from Red Rock collected in 1993 by Gutiérrez-Espleta et al. (2000; $N = 25$) and from 2009 reported in this study ($N = 27$). The first number in the Allele column indicates the alleles (size in base pairs) determined here in the 2009 sample and the numbers in parentheses indicates alleles from Gutiérrez-Espleta et al. (2000). — indicates that an allele was not observed.

		Frequency		Heterozygosity (# alleles)	
Locus	Allele	1993	2009	1993	2009
FCB266	89 (87)	0.220	0.407	0.587 (3)	0.514 (3)
	— (91)	0.200	—	—	—
	97 (—)	—	0.018	—	—
	101 (99)	0.580	0.574	—	—
FCB11	124 (127)	0.760	0.815	0.372 (2)	0.307 (2)
	126 (129)	0.240	0.185	—	—
MAF65	116 (115)	0.479	0.426	0.509 (2)	0.539 (3)
	124 (123)	0.521	0.537	—	—
	128 (—)	—	0.037	—	—
MAF209	112 (113)	0.580	0.426	0.577 (3)	0.632 (3)
	118 (119)	0.280	0.426	—	—
	120 (121)	0.140	0.128	—	—
MAF36	90 (93)	0.960	0.852	0.078 (2)	0.268 (3)
	98 (—)	—	0.074	—	—
	100 (—)	—	0.074	—	—
	— (109)	0.040	—	—	—
MAF48	123 (122)	0.340	0.130	0.606 (3)	0.350 (3)
	127 (126)	0.520	0.796	—	—
	129 (128)	0.140	0.074	—	—
FCB304	136	0.920	0.870	0.150 (2)	0.230 (2)
	140	0.080	0.130	—	—
MAF33	124 (—)	—	0.167	0.000 (1)	0.313 (3)
	126 (123)	1.000	0.815	—	—
	130 (—)	—	0.018	—	—
Mean	—	—	—	0.360 (2.25)	0.394 (2.75)

Table 3. The frequencies of alleles and heterozygosity (number of alleles) at eight microsatellite loci in the two studies of desert bighorn sheep *Ovis canadensis* from Tiburon by Hedrick et al. (2001; $N = 14$) and from Pilares in this study ($N = 10$). The first number in the Allele column indicates the alleles (size in base pairs) determined here in the 2009 sample and the numbers in parentheses indicates alleles from Hedrick at al. (2001). — indicates that an allele was not observed.

		Frequency		Heterozygosity (# alleles)	
Locus	Allele	Tiburon	Pilares	Tiburon	Pilares
FCB266	89 (87)	0.250	0.200	0.389 (2)	0.337 (2)
	101 (99)	0.750	0.800	—	—
FCB11	126 (127)	0.250	0.300	0.389 (2)	0.442 (2)
	128 (129)	0.750	0.700	—	—
MAF65	116 (115)	0.071	0.050	0.469 (4)	0.537 (4)
	118 (117)	0.036	0.050	—	—
	124 (123)	0.714	0.650	—	—
	130 (129)	0.179	0.250	—	—
MAF209	— (109)	0.107	—	0.619 (4)	0.626 (4)
	112 (113)	0.536	0.300	—	—
	114 (115)	0.036	0.100	—	—
	118 (119)	0.321	0.550	—	—
	120 (—)	—	0.050	—	—
MAF36	90 (93)	0.821	1.000	0.321 (3)	0.000 (1)
	— (107)	0.107	—	—	—
	— (109)	0.072	—	—	—
MAF48	123 (122)	0.286	0.050	0.424 (2)	0.100 (2)
	127 (126)	0.714	0.950	—	—
FCB304	136	0.179	0.050	0.601 (3)	0.100 (2)
	138	0.250	—	—	—
	140	0.571	0.950	—	—
MAF33	126 (123)	0.536	0.450	0.516 (2)	0.574 (3)
	128 (—)	—	0.050	—	—
	130 (127)	0.464	0.500	—	—
Mean	—	—	—	0.466 (2.75)	0.340 (2.50)

used. For instance, we have found that the size of a locus can change two base pairs after a change in the fluorescent dye label or two base pairs following shifting PCR amplification to the Qiagen PCR Multiplex Mix from our own PCR recipe. Within a laboratory, the solution to this variation is always to include a positive control that is a prior sample already genotyped. The usual solution for aligning alleles between laboratories is to run multiple samples in both to determine the correction factor for each locus. What should be consistent between labs is the spread of allele sizes and the distances between them for the same population. This should then allow allele alignment between labs without running samples in both labs.

As discussed above, for the eight loci analyzed both in the earlier samples from Red Rock by Gutiérrez-Espeleta et al. (2000) and Tiburon by Hedrick et al. (2001) and in this study from Red Rock and Pilares, allele size was consistently different by a few base pairs. For any given locus, this difference was always the same for all the alleles found. For example, for locus FCB266, the alleles in this study are two base pairs larger than in the previous studies (see Tables 2 and 3). In other words, because of the different molecular approaches used in the two laboratories here, such a small and consistent difference is not unexpected and resulted in unambiguous allele assignment.

For Pilares and 2009 Red Rock samples analyzed in this study, there were no discrepancies between results from the replicate PCRs run for each sample. One ram of the 28 Red Rock samples from 2009 was clearly an immigrant (discussed later) and was excluded in the analyses below. At 7 of 18 loci examined, he had alleles not otherwise found in the Red Rock sample, 4 of which were in homozygous state.

For the remaining 27 samples, none of the 18 loci deviated from Hardy–Weinberg expectations ($P = 0.15$–1.00). For the Pilares sample, two loci (MAF209 and

FCB193) out of 18 showed significant ($P \leq 0.05$) deviations from Hardy–Weinberg proportions; but with Bonferroni correction for multiple comparisons, no loci showed deviations. Similarly, for the Red Rock data from Gutiérrez-Espeleta et al. (2000) and the Tiburon data from Hedrick et al. (2001), no loci showed statistically significant deviations from Hardy–Weinberg proportions.

Comparison of 1993 and 2009 Red Rock samples

For the eight loci examined in both samples of bighorn sheep from Red Rock, genetic variation was somewhat higher in 2009 (this study) than in 1993 (Gutiérrez-Espeleta et al. 2000; Table 2) with the average heterozygosity increasing from 0.360 to 0.394 (9.4% higher but not significantly different) and the average number of alleles increasing from 2.25 to 2.75 (not significantly different). Four of these loci (FCB11, MAF209, MAF48, and FCB394) had the same alleles in both studies but in somewhat different frequencies. For three other loci, there was a gain overall of five alleles: a new MAF65 allele 128 at frequency 0.037; two new MAF36 alleles 98 and 100, both at frequencies of 0.074; and two new MAF33 alleles 124 and 130 at respective frequencies of 0.167 and 0.018 (Table 2). Additionally, for locus FCB266, both samples had three alleles with the middle allele in the 1993 sample four base pairs smaller than the middle alleles in the 2009 sample. Overall, the average frequency of alleles recorded in the 2009 sample but not in the 1993 sample was low at 0.048. Two alleles, FCB266-91 and MAF 36-109 appear to have been present in the 1993 sample, but not in the 2009 sample. The genetic distance between the 1993 and 2009 samples was 0.034 and was not statistically different from 0.

Variation in Tiburon and Pilares

For the eight loci analyzed for the Tiburon (Hedrick et al. 2001) and Pilares samples (this study), average heterozygosity was 0.466 for Tiburon and 0.340 for Pilares (27.0% lower and statistically significantly different; $P \leq 0.05$; Table 3). This reduction in heterozygosity was driven by substantial differences in heterozygosity at three loci (MAF36, MAF48, and FCB304) while the differences in heterozygosity at the other five loci were small. The average number of alleles per locus for the Tiburon sample was 2.75 compared with 2.50 for Pilares (not significantly different; Table 3). For these loci, the Pilares sample had lower heterozygosity than both the Red Rock (Gutiérrez-Espeleta et al. 2000) and Tiburon samples. The genetic distance between the Tiburon and Pilares samples was 0.051 and was not statistically different from 0.

Four of these loci had the same alleles in common in both samples (Table 3). The Tiburon sample had four alleles not found in the rams from Pilares, with frequencies of 0.107 (MAF209—109), 0.107 (MAF36—107), 0.072 (MAF36—109), and 0.250 (FCB304—138) for an average frequency over eight loci of 0.067. The Pilares sample had two alleles not found in the Tiburon sample, with frequencies of 0.050 (MAF209—120 and MAF33—128) for an average of 0.012 over eight loci. In other words, Tiburon had a higher frequency of alleles not found in Pilares than vice versa and the alleles found only in Pilares were in low frequency. As a result of the reduction in genetic variation and the loss of alleles, the Pilares rams appear to be a sample of sheep descended primarily from Tiburon. The two alleles found in Pilares but not in Tiburon could be because the 1998 sample of 14 individuals did not detect them or because the population from Sierra Punta Cirios contributed these alleles to the Pilares population.

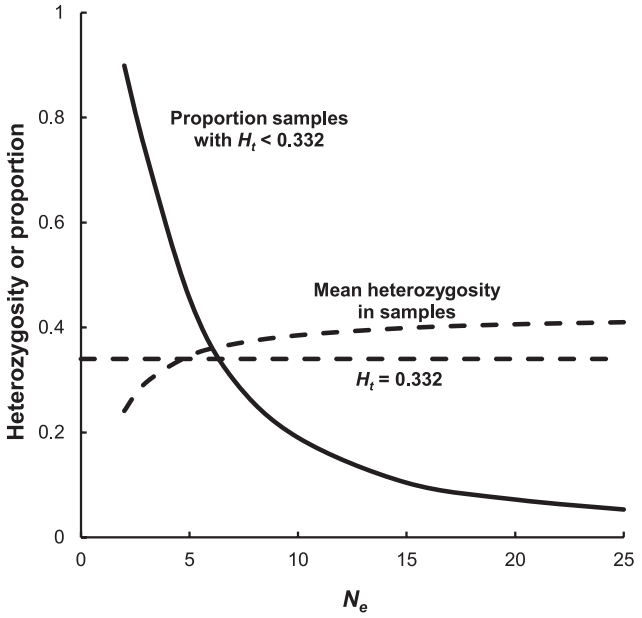

Figure 2. The average uncorrected heterozygosity (broken line) over 10,000 random samples for different effective population sizes (N_e) of desert bighorn sheep *Ovis canadensis* in two generations for the Tiburon–Pilares population and the straight broken line indicates the uncorrected observed heterozygosity in the Pilares sample for comparison. The proportion of the samples that have an uncorrected heterozygosity equal to or below that observed is also given (solid line).

Assuming that genetic drift was the major factor influencing genetic variation in this population, then the heterozygosity in the Pilares sample expected to be generated by genetic drift can be calculated. First, using equation (1) and assuming $H_0 = 0.466$, $H_t = 0.322$, $t = 3$, $N_{e.3} = 10$, and $N_{e.1} = N_{e.2} = N_e$, then $N_e = 3.4$. In other words, on average to have this decrease in heterozygosity, the effective population size in the two generations between the 1998 and 2010 samples, would need to be quite small. For example, if there were only one male successfully breeding each generation, the effective number is $N_e = 4N_f/(N_f + 1$; Hedrick 2011). Assuming that $N_e = 3.4$, then the effective number of females is 5.67. Assuming that $N_{e.3} > 10$, this would result in the estimate of N_e being even lower.

Using simulation, the proportion of samples for which the heterozygosity is equal to or below that observed can be calculated. Figure 2 gives this proportion for a range of effective population sizes in the two generations. For example, when $N_e = 4$, 37.8% of the samples had a heterozygosity equal to or below that observed. In fact, even for an effective size of 10, 6.9% of the samples

Table 4. The frequencies of alleles and heterozygosity (number of alleles) at 10 additional microsatellite loci reported in this study of desert bighorn sheep *Ovis canadensis* from the Red Rock population (N = 27) and from the Pilares population (N = 10).

		Frequency		Heterozygosity (# alleles)	
Locus	Allele	Red Rock	Pilares	Red Rock	Pilares
AE16	85	0.315	0.200	0.729 (6)	0.590 (3)
	91	0.241	0.200	—	—
	93	0.352	0.600	—	—
	95	0.037	—	—	—
	97	0.018	—	—	—
	105	0.037	—	—	—
TGLA387	139	—	0.100	0.560 (3)	0.595 (4)
	145	—	0.050	—	—
	147	0.444	0.250	—	—
	149	0.056	—	—	—
	151	0.500	0.600	—	—
HH62	110	0.093	—	0.731 (5)	0.710 (4)
	114	0.056	—	—	—
	116	0.278	0.200	—	—
	118	—	0.100	—	—
	122	0.167	—	—	—
	128	—	0.350	—	—
	130	0.407	0.350	—	—
JMP29	128	—	0.100	0.406 (4)	0.674 (4)
	130	—	0.300	—	—
	132	0.111	0.100	—	—
	134	0.759	0.500	—	—
	136	0.111	—	—	—
	144	0.018	—	—	—
FCB193	103	0.167	0.150	0.314 (3)	0.426 (3)
	105	—	0.100	—	—
	111	0.815	0.750	—	—
	115	0.018	—	—	—
AE129	167	0.315	0.250	0.639 (4)	0.768 (4)
	169	0.148	—	—	—
	171	—	0.350	—	—
	177	0.037	—	—	—
	179	0.500	0.250	—	—
	185	—	0.150	—	—
TCRBV62	170	0.056	—	0.661 (4)	0.442 (2)
	174	0.500	0.300	—	—
	176	0.222	0.700	—	—
	180	0.222	—	—	—
MMP9	188	0.056	—	0.616 (5)	0.653 (3)
	192	0.018	—	—	—
	194	0.185	0.500	—	—
	196	0.167	0.300	—	—
	198	0.574	0.200	—	—
BL4	157	0.593	0.900	0.544 (4)	0.190 (2)
	159	0.056	—	—	—
	161	0.018	0.100	—	—
	163	0.333	—	—	—
OMHC1	188	0.889	0.650	0.201 (2)	0.479 (2)
	192	—	0.350	—	—
	196	0.111	—	—	—
Mean (using 18 loci from Tables 2–4)				0.458 (3.44)	0.475 (2.83)

had a heterozygosity equal to or below that observed. In other words, even though it appears likely that the effective population size in these two generations was quite small, it is possible that it might have been somewhat larger.

Comparison of Pilares and Red Rock

For 18 loci the average heterozygosities of 2009 Red Rock and 2010 Pilares samples analyzed in this study are nearly identical (0.458 and 0.475, respectively; Table 4). There are more alleles per locus in the Red Rock sample (3.44) than in the Pilares sample (2.83), suggesting that small population size has been important in reducing the number of alleles in the Pilares population (Table 4). There are many alleles in the Red Rock sample that are not in the Pilares sample and vice versa; for all 18 loci (Tables 2–4), 26 of the 62 alleles (41.9%) in Red Rock are not found in the Pilares sample and 15 of the 51 alleles (29.4%) in the Pilares sample are not found in the Red Rock sample. The average frequency per locus over all 18 loci of alleles found in Red Rock but not in Pilares is 0.153, while the average frequency per locus of alleles found in Pilares but not in Red Rock is 0.175. The genetic distance between these two samples is considerable at 0.297, and statistically significantly different ($P \leq 0.05$).

What might be the effect of combining the Red Rock and Pilares populations on the amount of heterozygosity? Beginning with 2011 count data of 10 Pilares rams, 27 rams for Red Rock, and 38 Red Rock ewes (E. Rominger, personal communication), PI = 0.133 and PI' = 0.266. Because the heterozygosities are very similar for the two populations, adding the Pilares rams before breeding does not significantly change the average heterozygosity (Table 5). However, when the heterozy-

Table 5. The initial average expected heterozygosity (H_E) when the desert bighorn sheep *Ovis canadensis* samples in this study are combined with the proportion (PI) from Pilares before breeding, the observed heterozygosity (H_0) in the progeny of the first generation assuming the proportion PI' of males from Pilares, and the expected heterozygosity in the second generation (H'_E) over 18 loci. These values are not corrected for small sample size.

PI (population)	PI' (males)	H_E	H_0	H'_E
0.5	1.0	0.452	0.593	0.522
0.25	0.5	0.459	0.529	0.518
0.133	0.266	0.462	0.500	0.495
0.0	0.0	0.466	0.466	0.466

gosity in the progeny is calculated (H_0) using equation (3), the heterozygosity is significantly increased when the proportion of Pilares males is substantial. For example, if there were only Pilares rams, the value of H_0 is 0.593, 27.3% higher than with no Pilares rams. Using the estimate of Pilares rams in 2011, the increase is only to 0.500, 7.3% higher (Table 5). Although these are temporary heterozygosity excesses that will not persist, there is nevertheless the potential for a longer term heterozygosity increase measured by expected heterozygosity (H'_E in Table 5). For example, if all rams are from Pilares, this value is 0.552, and if $PI = 0.133$ (the estimate from 2011) then H'_E is 0.495.

Discussion

The genetic data examined here allow both an examination of the change in genetic variation over several generations in two populations and the potential impact of the introduction of individuals from one population into the other. The Red Rock population exhibited an unexpected increase in genetic variation over about three generations, while the Tiburon–Pilares population showed a large decrease in genetic variation over about two generations. These two populations both have low genetic variation compared with other desert bighorn sheep populations (Gutiérrez-Espeleta et al. 2000; Hedrick et al. 2001; J. Wehausen, unpublished data); but, because they are significantly different from each other, introduction of Pilares sheep into the Red Rock population has the potential to produce some increase in genetic variation.

Comparison of 1993 and 2009 Red Rock samples

If genetic drift was the only factor important in this population, genetic variation would be expected to decline over time between samples, and the number of alleles found only in the later sample would be less than that found in the earlier sample. However, because heterozygosity and the number of alleles observed in 2009 are higher than was observed in 1993, other factors than genetic drift appear to have dominated genetic change in this population during this period.

First, there might have been differences in the laboratory methods used or in chance sampling differences in the two samples that resulted in more alleles being identified in the later sample. However, there were no discrepancies between replicates run for the 2009 sample including loci also run for the 1993 sample. Further, for 55 Rocky Mountain bighorn run as part of the study by Gutiérrez-Espeleta et al. (2000), no discrepancies with genotypes obtained by Forbes et al. (1995) for the same samples were found. In addition, common genotyping errors (allelic dropout, false alleles, null alleles) often produce departures from Hardy–Weinberg proportions, but no loci in either sample differed from Hardy–Weinberg. In addition, by chance some alleles might not have been included in the 1993 sample but might have been seen in the 2009 sample or vice versa. The 1993 and 2009 samples were similar in size (25 and 27, respectively) and large enough that it is unlikely that chance sampling differences would have detected six alleles in the 2009 sample not found in the 1993 sample, while only two alleles detected in the 1993 sample were not found in the 2009 sample.

Second, because the two samples were obtained 16 y (approx. 3 bighorn sheep generations) apart, there is the possibility that allele frequencies could also have changed from mutation or gene flow. Because the new alleles found in 2009 were in low frequency, some could have been generated by mutation, low levels of gene flow, or chance sampling effects as discussed above. One of these alleles differed by two nucleotides from other alleles (the most common type of mutation for dinucleotide microsatellite loci), four only differed by four nucleotides, and one differed by six nucleotides. In populations of captive Gila topminnows *Poeciliopsis occidentalis*, a number of new alleles ($\geq$5) appear to have been generated by mutation at microsatellite alleles over just 5 y (about 10 generations; Hedrick et al. 2012).

In 2007, three rams appeared outside the fence at the Red Rock facility. After one of these rams was established genetically to be a desert bighorn sheep, all three were allowed into the refuge based on the assumption that they were Red Rock sheep that had escaped. The immigrant that we detected in the 2009 sample was probably one of those rams, which are now suspected to have come from Arizona (E. Rominger, personal communication). Although these rams may have successfully bred with Red Rock ewes, there appears to have been insufficient time for their breeding to have affected allelic variation in our 2009 sample that consisted of rams born prior to 2008.

Variation in Tiburon and Pilares

The 48 sheep used to start the Pilares population came from Tiburon, two different populations created from Tiburon sheep, and one native population from nearby mainland Sonora. We found much lower heterozygosity in our sample of the Pilares population compared with that found in the 1998 Tiburon sample and suggested that this was the result of small effective population size in the intervening period. This is also significant because the sheep introduced to Pilares from the one native Sonora population would be expected to increase genetic variation. Significant small population size effects that might reduce genetic diversity at Pilares could include founder effects for the three populations established from Tiburon Island sheep that were used as Pilares founders and possible high variation in the genetic contributions of different individual sheep (particularly rams) at various stages, greatly reducing effective population size values; but those details are unknown.

Bailey (1990) expressed concern about potential genetic effects of what he termed dilution transplants: founding of a population through translocation of a limited number of bighorn sheep from a native herd and then using that re-established population as the source of sheep translocated to create another population; and even using that third population as a source of stock for

a fourth population (double-dilution transplants). In essence, this is what appears to have occurred with the creation of the Pilares population and its use now as a source of many sheep moved to re-establish populations in vacant habitat in Mexico. Our findings relative to the significant decline in genetic diversity in our sample from Pilares compared with Tiburon Island two generations earlier supports the concern of Bailey (1990). It is also likely that the Tiburon sample from 1998 would have shown a reduction in genetic diversity relative to its source population in Sonora. The effect of this change in genetic variation on fitness in the Pilares sheep, both those translocated and those released in the wild, is unknown.

Combining Pilares and Red Rock bighorn sheep populations

As we discussed, introduction of Pilares ancestry into the Red Rock population could increase the level of genetic variation in the Red Rock population. However, when the 10 rams from Pilares were introduced into the Red Rock population, there were still 27 Red Rock rams, some of which were large and dominant, potentially limiting the breeding success of these 10 rams in the first year (2011). By the second year (2012), 4 of the Pilares rams had died, while 21 Red Rock rams were still present, further limiting potential genetic infusion. If the genetic profile of the immigrant ram sampled in 2009 is representative of the other two with him in 2007, their release into the Red Rock pen might also have influenced genetic population structure. After genetic data on the immigrant sampled in 2009 were developed, New Mexico Department of Game and Fish personnel recaptured him and returned him to the Red Rock population; but he subsequently died, probably before contributing more genes. However, one of the three immigrants from 2007 remains in the Red Rock population.

Interestingly, the sheep that were used to establish the Tiburon Island (Pilares) population in 1975 were from near Punta Chueca, Sonora, and the Mexican sheep that were brought to Red Rock in 1972 were from near Pico Johnson, Sonora (W. Montoya, personal communication). These animals appear to be from the same population because the areas near Punta Chueca and near Pico Johnson appear to be in the same general area, at most about 20–30 km apart. As a result, it is likely that most of the differences between the Pilares and Red Rock populations are the result of ancestry in the Red Rock population from the San Andres Mountains.

To provide a perspective on the potential for genetic rescue in this situation, it is useful to examine genetic rescue in other situations. First, in the Florida panther *Puma concolor coryi*, it was decided that initially approximately 20% of the ancestry in the genetic restoration effort would be from Texas cougars because this allowed detrimental variants to be selected against and adaptive variants to be retained (Hedrick 1995). Eight females were introduced (estimated to be about 20% of the population), and the five that reproduced were very successful and appear to represent a high proportion of the females reproducing (Johnson et al. 2010). In fact, this high success might swamp the Florida panther ancestry (Hedrick 2010).

Second, two captive lineages of Mexican wolves *Canis lupus baileyi* (Ghost Ranch and Aragon) were combined with the primary captive lineage (Certified, now known as McBride) starting in 1996 (Hedrick et al. 1997). The initial recommendations were that only 10% of the ancestry should be from each of Ghost Ranch and Aragon. Although crosses between these lineages had high fitness (Fredrickson et al. 2007), because of high levels of human-caused killing and management problems, the proportion of ancestry has not increased beyond these initial goals. Finally, in two cases, natural introductions of single male wolves greatly changed the ancestry in wild wolf populations. In Sweden, the introduction of a male wolf from the Finland–Russian population around 1990 into a population of about 10 resulted in genetic (or behavioral) rescue and the population greatly increased (Vilà et al. 2003). In the Isle Royale wolf population, a single male migrant from Canada in 1997 was so successful that 56% of the ancestry was from him and 34% was from his first mate (Adams et al. 2011).

General Implications

Analysis of the Red Rock population over time and of the Tiburon–Pilares population demonstrated the difficulty of predicting temporal change in genetic variation. Genetic variation apparently increased in the Red Rock population, where there have been management efforts to keep the Red Rock population size large enough to reduce loss of genetic variation. On the other hand, genetic variation decreased in the Tiburon–Pilares population, where it is unclear that there have been management efforts to maximize the effective population size. Further, the introduction of Pilares rams (and the probable Arizona ram) into Red Rock might further increase the amount of genetic variation. Although there is no direct evaluation of fitness characteristics in these populations, if a lower level of genetic variation results in a consequent lower fitness and less ability to cope with environmental challenges (such as diseases and climate change), then the Pilares population and its derivative populations may be vulnerable. In other words, from the information here, it is likely that the Pilares population has been well below the effective population size level of 50, suggesting that it is likely to have impacts from inbreeding depression.

The examples of genetic rescue discussed above illustrate that the success of genetic rescue might be very case-dependent, sometimes resulting in a complete turnover in ancestry of the population (Florida panther and wolves in Sweden and on Isle Royale). Based on these cases, Hedrick and Fredrickson (2010) suggested guidelines that could be used for genetic rescue, and examination of these guidelines in the case of the Red Rock desert bighorn sheep population might be insightful. Because the introduction of Pilares rams into Red Rock is at a very early stage, it is not yet clear how much of the Pilares ancestry will be incorporated into the

Red Rock population. The proportion of Pilares rams might be adequate if they are reproductively successful. Examining the paternity in lambs born since the introduction of the Pilares (and the Arizona) rams should give an initial indication of the success of this introduction. Determining the impact of fitness is more difficult because there has been little baseline analysis of fitness components, such as individual viability and reproductive success, although analysis of some past data is possible.

The impact of introductions to increase genetic variation can have the most impact in several situations. First, when the population is small, either at its initiation or during a natural or artificial bottleneck, the level of ancestry from the donor population can have a greater effect because of relative numbers. On the other hand, if the population size is large, then to have the same impact on ancestry level, the number of introduced individuals must be substantially higher. Second, the impact of an introduction might be significantly reduced if there is a dilution effect caused by sequential founding of populations from small numbers of individuals. It appears that the Pilares population might have experienced this effect and consequently have lowered genetic variation and possibly lowered fitness. Third, when there is low fitness in the population, then the ancestry from the introduced individuals might increase very quickly because of differential selection. This was apparently the case for Florida panthers, wolves in Sweden and on Isle Royale, and bighorn sheep on the National Bison Range (Hogg et al. 2006; Miller et al. 2012). How the Red Rock bighorn sheep population fits into this scenario is uncertain at this point, but further examination of the population over time would be important.

Supplemental Material

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Table S1. Primer sequences and references for the 18 microsatellite loci run for 2009 Red Rock and 2011 Pilares samples of desert bighorn sheep *Ovis canadensis*.

Found at DOI: 10.3996/082013-JFWM-055.S1 (55 KB DOC).

Table S2. Genotype data for the 27 Red Rock and 10 Pilares samples used in analyses.

Found at DOI: 10.3996/082013-JFWM-055.S2 (192 KB DOC).

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