Genetic Bottlenecks Resulting from Restoration Efforts: The Case of Bighorn Sheep in Badlands National Park

Rob Roy Ramey II[1,4]
Gordon Luikart[2]
Francis J. Singer[3]

Abstract

Using the example of a reintroduced bighorn sheep population in Badlands National Park, South Dakota we demonstrate the usefulness of neutrality tests and demographic data for detecting a severe genetic bottleneck (Ne < 10). From demographic data the effective population size of the founding population at Badlands was estimated to be six, and a heterozygosity excess test revealed evidence of a severe population bottleneck. We discuss the criteria for intervention when there is evidence of a severe bottleneck, and propose methods of mitigating the potentially deleterious long-term consequences of such bottlenecks. These issues are presented in the context of bighorn sheep reintroductions, but the issues are also of general importance to restoration efforts involving other large vertebrates.

Key words: genetic bottleneck, bighorn sheep, *Ovis canadensis*, reintroduction, heterozygosity excess, microsatellites.

[1]Department of Zoology, Denver Museum of Nature and Science, 2001 Colorado Blvd., Denver, CO 80205–5798, U.S.A.
[2]Laboratoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Universite Joseph Fourier, F-38041 Grenoble Cedex 09, France
[3]U.S. Geological Survey, Midcontinent Ecological Science Center, Natural Resources Ecology Lab, Colorado State University, Fort Collins, CO 80525, U.S.A.
[4]Address correspondence to R. R. Ramey, email ramey@colorado.edu

Introduction

Severe demographic and genetic bottlenecks can sometimes occur as a result of conservation actions, such as the reintroduction of animals into former parts of their range (McCullough et al. 1996). The implications of a severe genetic bottleneck may not be readily apparent in the time scale of most management programs, because few generations have elapsed since the original translocation or other factors have obscured the effects of inbreeding.

Translocations have been used as the primary management tool for reestablishing bighorn sheep (*Ovis canadensis*) populations in the western United States, Canada, and Mexico since 1921 (Ramey 1993). The number as well as sex and age composition of animals introduced in these translocations has varied widely (Bailey 1990; Ramey 1993). The overall rate of successfully establishing self-sustaining populations has been approximately 50%. Recent analysis of translocation success (Singer et al. 2000*a*) suggests a positive correlation between the number of individuals translocated and the maximum recent population size. It is generally accepted that demographic stochasticity in small populations often plays a greater role than genetics in the decline or loss of some translocated populations (Lande 1988). However, it is prudent to avoid severe genetic bottlenecks (e.g., effective population size [Ne] of less than 10; Lande 1994) in translocated populations, especially if these newly established populations cannot receive new variation from other outbred populations via gene flow. Furthermore, several recent publications have suggested that the genetic changes caused by population bottlenecks can substantially increase the risk of population extinction due to the deleterious effects of inbreeding and increased susceptibility to disease (O'Brien & Everman 1988; Lande 1994; Mills & Smouse 1994; Newman & Pilson 1997; Saccheri et al. 1998; Westemeier et al. 1998).

Because of its excellent documentation, we chose the restoration of bighorn sheep into Badlands National Park, South Dakota as a case study to illustrate several restoration issues important to the long-term conservation of bighorn sheep. These issues include methods that can be used for detecting severe population bottlenecks, criteria for intervention in bighorn sheep population bottlenecks, and management alternatives for mitigating the potentially deleterious long-term consequences of severe population bottlenecks.

In 1964 a total of 20 bighorn sheep was initially translocated from Pikes Peak, Colorado to a 150 ha enclosure in Badlands National Park, South Dakota. After a 50% loss in the number of bighorn in this enclosure due to *Pasturella* infection, a total of 14 animals (two adult rams, two adult ewes, four yearling ewes, three ram lambs, and three ewe lambs) were released into the

wild in August 1967. These animals established a self-sustaining population that grew to an estimated high of 140 in 1988 in two subpopulations (Singer & Gudorf 1999) and declined to approximately 60 animals by 1996. In 1981, eight animals from the North Unit of Badlands colonized the South Unit of Badlands National Park, establishing the second subpopulation. In 1982, a second disease epizootic apparently caused by an outbreak of bluetounge and/or *Pasturella* had reduced the population in the North Unit to about 50–60 animals. A third disease epizootic, beginning in the early 1990s, had reduced the total Badlands population to approximately 60 animals by 1996. In 1996 the North Unit population was used as the source for a translocation to a nearby area of habitat at Cedar Pass, involving 12 ewes and 4 rams, in an attempt to establish a third subpopulation. Both the South Unit and Cedar Pass subpopulations may now be linked by gene flow to the North Unit subpopulation.

Effective population size (Ne) is one of the most important parameters in evolution and conservation biology because it estimates the rates of inbreeding and loss of genetic variation in populations. This is because the census size of a population usually does not reflect the actual genetic contribution of individuals to future generations or the overall rate of inbreeding in a population. Ne also influences the efficiency of natural selection in maintaining beneficial alleles and purging deleterious ones (Lande 1994). For example, when Ne is very small, genetic drift can overide the effects of natural selection. Although it is possible to estimate effective population size using a variety of theoretical models, including sex ratios and data on fluctuations in census size of populations, accurate data on these parameters are often difficult to obtain from study populations in the wild, and often overestimate Ne (Harris & Allendorf 1989; Husband & Barrett 1992; Frankham 1995*a*; Frankham 1995*b*; Schwartz et al. 1998).

Traditional approaches for detecting genetic bottlenecks using comparisons of mean heterozygosity in native versus translocated populations lack resolution because they require large sample sizes, and have large confidence intervals surrounding estimates (Schwartz et al. 1998). Recently, advances in molecular population genetic theory have yielded a new class of analytical methods that are based on neutral models of molecular evolution to infer aspects of a population's demographic history from genetic data (Rand 1996; Luikart et al. 1998*a*; Luikart et al. 1998*b*). The most widely used tests to date include the heterozygosity excess test for allele frequency data from nuclear loci (Cornuet & Luikart 1996; Luikart & Cornuet 1997) and Tajima's test for sequence data from mitochondrial DNA (Tajima 1989; Rand 1996). The basis of these "neutrality tests" is a comparison of the observed molecular diversity in a population with that expected under a neutral model of molecular evolution, given a similar set of parameter values such as the number of alleles, their frequency distributions, and divergence among alleles. Monte Carlo simulations are then used to generate distributions against which to test the goodness-of-fit of the equilibrium model with the observed data.

For the Badlands population, we used the test of heterozygosity excess (Cornuet & Luikart 1996; Luikart & Cornuet 1997) on microsatellite and allozyme data from Buskirk and Johnson (1995) to determine if the Badlands bighorn sheep population shows the genetic signature of one or more substantial genetic bottlenecks such that current Ne is less than 10. When a population undergoes a genetic bottleneck, both the number of alleles (allelic diversity) and heterozygosity are reduced. However, allelic diversity is reduced faster than heterozygosity, leaving a transient deficiency in the observed number of alleles relative to the number of alleles expected from the observed heterozygosity. This method detects the loss of rare alleles in a bottlenecked population relative to that expected under mutation-drift equilibrium (neutrality) for an observed heterozygosity calculated from microsatellite and/or allozyme allele frequency data. We also used the mode shift test (Luikart et al. 1998*b*) to detect the distortion in the distribution of allele frequencies from many rare alleles to fewer, more common alleles when a severe genetic bottleneck occurs. The performance of this method has been evaluated using empirical data from a wide variety of bottlenecked and non-bottlenecked populations (Luikart et al. 1998*a*; Luikart et al. 1998*b*), and their behavior evaluated via computer simulations (Luikart 1998*b*).

Methods

The population history of the Badlands population come from the South Dakota Division of Wildlife (T. A. Benzon 1990, personal communication) and F. Singer (unpublished data). The genetic data were obtained from a survey of genetic variation in 26 bighorn from this population (Buskirk & Johnson 1995). That study revealed five polymorphic allozyme loci (Glo1, Ldh2, Mpi1, pp, and Trf) and four polymorphic microsatellite loci (TGLA137, TGLA188, TGLA427, and TGLA116) in the Badlands population. We used the analytical methods of Cornuet and Luikart (1996) to test for heterozygosity excess, as well as the computer program BOTTLENECK (Piry et al. in press) on a combined analysis of both microsatellite and allozyme data. The locus, allele frequencies, and sample size (number of individuals sampled) used in the analyses were: Glo1: 0.788, 0.212, $n = 26$; LDH2: 0.904, 0.096, $n = 26$; MP: 0.558, 0.442, $n = 26$; PP: 0.788, 0.212, $n = 26$; TRF 0.885, 0.115, $n = 26$;

TGLA137: 0.596, 0.404, $n = 26$; TGLA188: 0.923, 0.077, $n = 26$; TGLA427: 0.580, 0.320, 0.100, $n = 25$; and TGLA116: 0.479, 0.458, 0.063, $n = 24$. Analyses were first carried out on all loci and then for all loci except TGLA137 and TGLA188 because these showed significant deviations from Hardy-Weinberg expectations (and, therefore, could not be expected to fit the neutral expectations of the model). Three models of molecular evolution were considered, for this combined analysis: the infinite allele model (IAM) with each mutation producing a new allele different from others in the population; the stepwise mutational model (SMM) with mutation resulting in change one step forward or one step backward with equal probability and therefore fewer alleles than with the IAM; and the two-phased mutational model (TPM). All three models were considered because while allozyme loci generally fit an IAM, microsatellites are more likely to fit a SMM. The TPM is a mixed mutational model that allows for a specified percentage of SMM in the analysis. Under TPM, the percent of SMM tested were 70% and 90% with a variance in mutation lengths of 30% and 4%. Five thousand iterations were used and a Wilcoxon signed rank test was used as a test of significance (Cornuet & Luikart, 1996; Piry et al. in press). The distribution of allele frequencies was tested against the expected L-shaped distribution as expected under mutation-drift equilibrium (Luikart & Cornuet, 1997; Luikart et al., 1998*b*).

The initial effective population size for Badlands is estimated at six, counting only adults and yearlings and using the equation Ne = 4Nm*Nf/(Nm+Nf), where Nf is the number of breeding age females and Nm is the number of breeding age males. Assuming that all of these individuals survived and reproduced, the maximum founding effective population size would be 12.9.

Results

Using all loci, the test for heterozygosity excess showed significant deviations from neutral expectations for a stable population under the IAM ($p = 0.014$) and TPM for both sets of simulation parameters ($p = 0.019$ for variance = 30%, proportion of SMM in TPM of 70%; $p = 0.024$ for variance = 4%, proportion of SMM in TPM of 90%) but not the highly conservative SMM ($p = 0.102$). A significant mode shift was also found in the observed allele frequency distribution (i.e., proportion of rare alleles) to the expected L-shaped distribution for a large, non-bottlenecked population (which typically contains many rare alleles).

When TGLA137 and TGLA188 were dropped from the analysis, similar results were obtained with significant deviations from expectations for IAM ($p = 0.019$) and TPM for both sets of simulation parameters ($p = 0.019$ for variance = 30%, proportion of SMM in TPM of 70%; $p = 0.02$ for variance = 4%, proportion of SMM in TPM of 90%) and also for the highly conservative SMM ($p = 0.019$).

Discussion

From both the estimated effective population size (using the number and sex of breeding age founders) and the analysis of molecular genetic data, it is clear that the Badlands National Park population has undergone a serious population bottleneck at founding. The heterozygosity excess tests are sensitive to bottlenecks of Ne < 10 and the distribution of allele frequencies using mode shift test was approximately equivalent to that of a theorized population with an Ne = 4 lasting for eight generations (Luikart et al. 1998*b*). It is rare to have both demographic and genetic information from the same population to infer a population bottleneck.

Although we can infer that a genetic bottleneck has occurred, it is not yet apparent from direct observations alone that such a genetic bottleneck is deleterious in the Badlands population. This could be because environmental factors such as disease and predation, as well as demographic stochasticity, can obscure the population level effects of a genetic bottleneck. Also, the degree of mating among close relatives in small isolated populations is unknown. There is correlative evidence, however, that shows that the use of indigenous versus previously translocated populations (possibly with lower genetic diversity) as a source doubled the rate at which populations achieved a size greater than 100 (Singer et al. 2000*a*). Similarly, Singer et al. (2000*b*) in a separate study of 31 translocated populations, found that founder size was significantly associated with the later size of a translocated population and that mixing sources in the founder population also increased later population size.

Based on evidence of deleterious consequences of inbreeding from other animals and theoretical models (Mills & Allendorf 1996; Saccheri et al. 1998; Frankham 1995*a*; Frankham 1995*b*, 1999), it is justified to intervene when there has been a severe genetic bottleneck (e.g., Ne < 10), and a lack of gene flow with other outbred populations. Such an action may be termed "prudent intervention" because it is preemptive in nature.

There are five key issues that need to be addressed when considering the augmentation of populations such as the Badlands. First, are there two lines of evidence (e.g., genetic, demographic, and/or behavioral) that support the hypothesis that a severe population bottleneck has occurred? Second, would the introduction of additional animals degrade range conditions, driving them to a more rapid extinction? Third, was the population bottleneck(s) due to a disease outbreak, and can the source/vector of the disease be eliminated? Fourth, are there adequate habitat patches nearby to es-

tablish a metapopulation of larger size, rather than a single, isolated population? And fifth, how should the sex and age composition of an augmentation be structured?

The first question has been addressed from the analysis presented above using both demographic data and the heterozygosity excess test. We support the approach of using two lines of evidence to make a strong inference that a severe genetic bottleneck has occurred. A genetic test based on a single sample may not be adequate to detect a bottleneck. This is because sample sizes may not be adequate or the assumptions of the model may be violated by selection or a highly polygynous mating system (Schwartz et al., 1998). If demographic data are lacking, the temporal allele method (Waples 1989; Schwartz et al. 1998) may be used which relies on samples taken at two points in time. However, this method requires approximately 45 individual samples for 10 loci at two points in time to detect a Ne < 10 (Luikart & Cornuet 1997).

If both a demographic and detectable genetic bottleneck are observed in a population (e.g., an estimated Ne of 10–20) this should be clear grounds for intervention, such as adding additional animals to the population from a non-bottlenecked source. Although the population is currently at high N, low genetic variation could put it at risk (e.g., susceptibility to disease).

The second question can be addressed with a habitat utilization model to estimate the carrying capacity of the range. In the case of Badlands National Park, such a model (Singer & Gudorf 1999) has shown that the habitat can support as many as 400 bighorn sheep in five different subpopulations. In other cases, if the habitat is found to be inadequate, range conditions can often be improved to support more animals using such methods as controlled burns to improve habitat.

The third question concerns the causes of recent population declines, such as that which occurred in 1982, and how to prevent other disease-related declines in the future. Because bighorn sheep are susceptible to a variety of domestic livestock diseases (Foreyt & Jessup 1982; Goodson 1982; Jessup 1985; Onderka et al. 1988; Foreyt 1989), it is wise to have an ongoing monitoring program in place to identify the potential sources of disease, the parasite or pathogen when an outbreak does occur, and to determine ways to deal with the most likely outbreaks. Vigilance and preplanning of control measures can be seen as a preemptive strategy, commonly used in the human health infrastructure but rarely in wildlife conservation. In the case of Badlands National Park, the limited scale of the monitoring program failed to detect an approximately 50% decline in the population in the early 1990s and the cause of mortality, except in a few isolated cases (Singer et al. 2000*a*). Clearly, this is an area where there is room for improvement. Also, there is one remaining fenced domestic sheep grazing allotment of approximately 30 animals between the North and South Units. This clearly needs to be eliminated or double fenced before investment in an augmentation can be undertaken.

The fourth question is important because the establishment of single isolated populations without the potential for genetic links to other populations is of limited value, in that it lacks long term conservation perspective. We advocate the establishment of metapopulations to both maximize the potential for long term conservation of genetic diversity, allow for demographic stochasticity, and reestablish the natural metapopulation structure of bighorn sheep (Ramey 1995; Bleich et al. 1996; Boyce et al. 1999). This conservation strategy requires a substantially larger planning and resource commitment than has been traditionally used in the past. This strategy also requires that the open habitats between subpopulations that are potentially used as movement corridors remain free of barriers (e.g., fences) that would inhibit dispersal between populations. In the case of Badlands National Park, habitat evaluation has shown that there are five habitat patches that could support separate subpopulations of bighorn sheep with the potential for gene flow among them (Singer & Gudorf 1999). Fortunately, National Park Service policy minimizes the amount of development that can occur, so future barriers to movement will be minimal. However, this is likely to be an issue on lands outside of national parks. Once again, GIS-based habitat models can help prioritize land conservation and management in areas identified as potential bighorn movement corridors.

The last question addresses the issue of "What to do?" Augmenting the population with ewes is the most direct means of increasing population numbers. From a genetic perspective this would take longer to have an effect on the population than introducing rams, because rams can make an immediate genetic contribution via matings with multiple ewes. However, augmenting the population with ewes poses a smaller risk in terms of livestock diseases from domestic sheep in the area because reintroduced rams have been documented to wander greater distances (Bleich et al. 1990; Ramey 1993) and are, therefore, more likely to come into contact with domestic sheep. Bighorn rams may seek mating opportunities with domestic sheep, which will expose them to pathogenic strains of *Pasturella hemolytica* bacteria carried by domestic sheep. The recommended way to avoid contact between bighorn and domestic sheep is to have a sufficiently wide buffer between the species (>20 km) or secure double fencing to prevent nose–nose contact which can transmit *Pasturella* (Singer et al. 2000*a*). One of these actions must be completed prior to implementing an augmentation at Badlands

that includes rams. It may be far more efficient to buy out the domestic sheep allotment and retire it.

Based on our analyses of demographic and genetic data for Badlands National Park and discussion of the issues surrounding intervention in genetically bottlenecked translocations, we recommend a substantial (>30) mixed sex augmentation of bighorn sheep from an outbred native source population of Rocky Mountain bighorn sheep. A mixed sex augmentation would provide both an immediate and long term genetic benefit to the Badlands population. These animals should be used to both augment existing subpopulations and establish at least one new subpopulation within the park. Among the constraints that will need to be addressed are the availability of source populations in the Rocky Mountains and the willingness of those states to contribute surplus animals that can be used in their own restoration efforts. However, multiple smaller augmentations could be carried out over several years if there is a shortage of animals available for a single large augmentation.

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