

CHAPTER 5

CONCLUSIONS

Chapter 5: Conservation Relevance and Management Recommendations

Synthesis of Genetic Findings

D. pygargus has been restricted to the grasslands and coastal plains that lie within the boundaries of South Africa since its origin during the Pleistocene. The evolution of this antelope has been influenced by climatic fluctuations during this epoch along with its tribe members (Arctander et al. 1999). In turn, these periods of change have caused a division of the species into two allopatric groups approximately 1 - 2 million years ago. Moreover, the harsh conditions of the past geologic ice age plus the more recent impact of over-hunting appear to have had drastic effect on the demography of the species reducing the number of mitochondrial lineages to very few. The low level of genetic diversity found at the fast evolving loci (control region and microsatellites) suggests that this alcelaphine species was not as successful as its conspecifics. In contrast, extensive diversity was revealed within the MHC-DRB locus. This high level of DRB polymorphism is far greater than the diversity reported in any other exotic ungulate.

Conservation and Evolutionary Units for Taxonomy

The combination of genetic data can be applied to a conservation structure that is able to satisfy the viewpoints of all competing theories of conservation policy. Firstly, the data revealed strong genetic partitioning between bontebok and blesbok, which supports the recognition of as distinct taxa deserving, separate management. This line of evidence fulfils the systematic conservation perspective in that each taxon is an evolutionary distinct lineage. The conservation efforts would include the continued

management of each subspecies in their respective habitats and, most importantly, the restriction of hybridization. Secondly, the subspecies inhabit different ecosystems that support a diverse array of fauna and flora. The bontebok inhabit renoster shrubland that is part of the fynbos ecosystem of the western Cape. This ecosystem supports a unique kingdom of flora endemic to South Africa. The open grasslands that support blesbok populations throughout South Africa are disappearing at an alarming rate to support mono-culture agriculture crops, commercial forests and cattle farms. The negative consequences of grassland removal will result in the erosion of soil, desertification and extinction of endemic species within this ecosystem. The management of each subspecies in its ecosystem will in effect sustain the biological processes that support life. Lastly, the molecular results satisfy the criteria of evolutionary conservation to preserve genetic diversity of both subspecies. The genetic paucity of the species is evident from the complete monomorphism of mtDNA in bontebok and low amount of diversity in blesbok. Evolutionary theory predicts that genetic diversity permits a species to respond to stochastic changes. From the control region data, I predict that *D. pygargus* experienced demographic crashes possibly during both the Pleistocene and recent times.

Inbreeding Depression

Since the early 1980's, biologists have supported the genetic theory of inbreeding between closely related individuals leads to increased risk of extinction of the population. Inbreeding has been suggested to cause fixation of deleterious alleles and well as the loss of adaptive genetic variation (Lande 1988). Strong evidence from empirical studies of captive and laboratory populations (Ralls et al. 1979, Frankham et al. 1999) has correlated the cause of extinction in populations due to inbreeding depression. Moreover, the significance of inbreeding depression in wild populations has been demonstrated in song

sparrows (Keller et al. 1994) and butterflies (Saccheri et al. 1998). Uncovering a direct link between reductions in evolutionary potential within inbred mammal species, however, has been far more difficult to determine (Mikko et al 1999.)

Presently, conservation geneticists are still confronted with the contentious issue of relating depletion of genetic variation and species survival (Crmokrak and Roff 1999). The genetic data from this study clearly indicate a loss genetic diversity within bontebok at both nuclear and mitochondrial loci; however, there are no apparent signs of inbreeding depression in the present populations. Similar findings have been demonstrated in other mammalian species where inbred populations were able to recover, reproduce and flourish, despite low genetic variation (Hoelzel et al. 1993, Broders et al. 1999, Le Page et al. 2000).

Fitness traits

In order to relate the genetic variation of inbreeding depression to the phenotypic traits in a population, polymorphic neutral loci are chosen for analysis. It has been argued that since these genetic “markers” are non-coding, they are not a good indication of fitness (Caro et al. 1994). However, this argument does not consider the fact that inbreeding will reduce heterozygosity levels at the marker loci and other gene regions including those that control fitness traits.

Long-term behavioral and demographic studies of a species are needed in order to make assumptions about the relationship of genetic data to fitness traits of individuals. This present study focussed on only the genetic aspects of bontebok; however, I will attempt to describe the current population fitness measurements with respect to the genetic data.

The low molecular diversity uncovered within bontebok was expected given its demographic history. The subspecies' population size has remained relatively low compared to other ruminant species that have undergone severe bottleneck or founder events. For example, a population of moose in Newfoundland, Canada was founded by merely 6 individuals in the early 1900's and has since exploded to over 500,000 animals (Broders et al. 1999). Many factors including environmental conditions, lack of predators, stable sex ratios and reproductive success may have promoted the expansion of this moose population. According to the recent demographic history of bontebok, one or more of these factors may have prevented the growth of its population size.

It has been suggested that high inbreeding in animals may have a strong negative affect on sperm morphology and ejaculate quality (Roldan et al. 1999) and ultimately influence the success rate of fertilization. A cursory study of bontebok sperm morphology was reported by Skinner et al. (1980) in response to the low lambing rate within BNP. Unfortunately, this study was not a thorough investigation of sperm traits that would have led to a conclusion regarding fertilization success of bontebok rams (see Gomendio et al. 2000). Further sound reproductive studies may be able to link the genetic data with individual fitness so that the low lambing rate may be explained.

This investigation has provided a comprehensive genetic survey of bontebok that can be used to monitor populations for future management. Although, I have inferred that inbreeding has occurred within the subspecies, one cannot predict the effect which low genetic variation will have on the future evolutionary potential. The descendents of the bottleneck populations were able to survive and persist over time suggesting that the residual genetic variation was suitable to support viable populations. However, in the present, species are challenged with unnatural human mediated pressures such as habitat fragmentation, pollution, hunting and translocations. These new destructive forces create

a situation where the adaptive potential of the species is stretched to the limit, where genetic variation is crucial for long-term survival.

Hybridization Detection

Illegal translocation events have led to the hybridization between bontebok and blesbok subspecies. Although movements of animals are now tightly regulated between provinces, the initial translocation events have produced a large number of hybrids throughout South Africa. This has caused numerous problems in the conservation and management of both subspecies for conservation agencies. One of the objectives of bontebok conservation is to preserve the genetic integrity of the subspecies. In order to do so, the genetic purity of both bontebok and blesbok herds needs to be confirmed before animals can be moved between populations. Purity of bontebok herds located within national reserves and most private farms is verified by documentation. A discriminant analysis test of phenotypic traits has also been used to differentiate between hybrids and pure subspecies. In cases where there is doubt of classification, animals were tested by genetic markers.

Bontebok from two farms, Wag 'n Beitjie (WB=10) and Fairview (Bb=5) were analyzed for genetic purity using three classes of molecular markers. The control region was used to detect maternal introgression within each herd. The disadvantage of this method, of course, is that this mitochondrial gene is inherited maternally and would not pick up paternal introgression. The DRB gene provided private alleles for each subspecies and is a potential gene marker for detecting hybridization events from both sexes. Hybrids at the F1 level could readily be identified using only one marker, however, hybridization within further generations (F2, F3, ...) would require a panel of

genetic markers to uncover admixture. In this regard, I used a set of polymorphic microsatellite loci with relatively high heterozygosity and F_{st} values to detect possible hybrids. A large proportion of private alleles were found within each subspecies. These allele frequencies were used in two methods of classification. Individuals were assigned to subspecies designations based on each multilocus genotypes. The methods of classification included a clustering analysis and a likelihood approach. In both cases, suspected hybrids were placed within the bontebok category.

The results from all WB bontebok samples indicate genetic purity. The microsatellite analysis picked up a few rare alleles that were not found within either subspecies. However, the microsatellite test was not able to accurately detect hybridization within the Bb samples or correctly classify test animals in simulation tests. Two blesbok haplotypes were found with the population, clearly indicating maternal introgression. The DRB genotype of Bb3 revealed alleles not found within bontebok, which may be blesbok specific.

The small sample size of the test group is problematic in determining the strength of the genetic tests for hybrid detection. Many of the bontebok specific alleles occurred at low frequencies while alleles shared between subspecies were found at high frequencies. The large reduction of bontebok N_e may have caused the loss of potentially private alleles. Additional microsatellite loci could enhance the power of hybrid detection. Furthermore, microsatellites linked to coding genes may show a greater degree of fixation than the neutral evolving microsatellite markers.

Management Recommendations

I have identified two evolutionary lineages of *D. pygargus* and recommend that each be managed separately. The genetic evidence reported here supports the

classification of subspecies as units for conservation. This suggestion is based in part from the genetic evidence that confirms that they are historically isolated and evolve independently from one another. Furthermore, the DRB data demonstrated possible adaptive significance between the different subspecies specific alleles. In this regard, conservation of each subspecies will maintain the ecological and evolutionary processes of both bontebok and blesbok.

The genetic diversity remaining in bontebok should be conserved for future evolutionary change. In this regard, efforts should focus on enhancing population numbers as well as genetic exchange between existing populations. The controversy still remains as to how large a population size must be in order to preserve evolutionary potential. Results of some quantitative genetic studies have shown that small populations (100 individuals) are extremely susceptible to fixation of deleterious alleles and loss of adaptive potential. In addition, these studies suggest that a minimum of 1,000 breeding individuals is needed to avoid rare deleterious mutations from fixating in the population. The total population size should be one order of magnitude greater than the effective population size (N_e). In general, an (N_e) of approximately 500-1,000 individuals would allow for retention of evolutionary potential (Franklin and Frankham 1998). The present population size of bontebok is 2,500-3,000, which is well within the projected limit.

Biodiversity in South Africa

The successful recovery of bontebok is shadowed by the loss of another endemic South African antelope species called the blue antelope (*Hippotragus leucophaeus*), which went extinct in 1800. It has been suggested that changes in climate during the late Pleistocene reduced viable grasslands in the cape region for the blue antelope and

bontebok (Klein 1983). More recently, habitat destruction before European settlement may also have attributed to their decline (Robinson et al 1996). From the historical data, it appears that both antelope lived in similar conditions and experienced severe reductions in population size. Fortunately, the bontebok was saved from extinction and continues to contribute to the biodiversity of the cape region.