

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

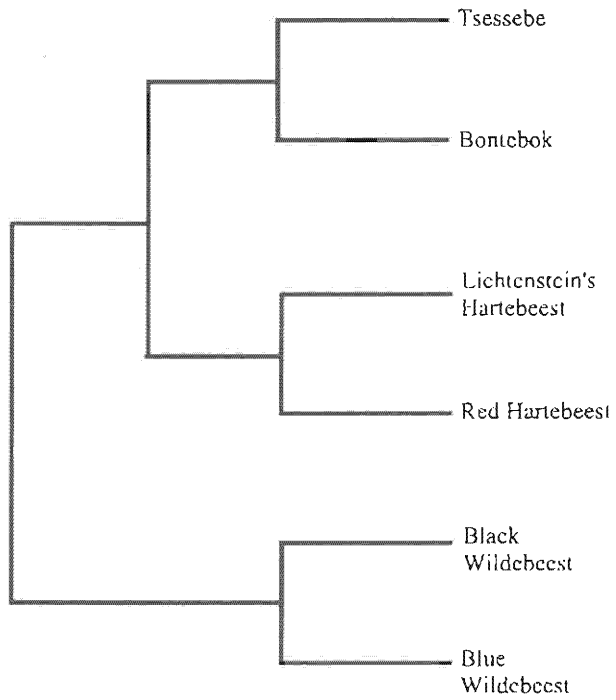
Chapter 1: Natural History of *Damaliscus pygargus* (Pallas, 1767)

Evolution of Damaliscus (Artiodactyla: Bovidae)

The family Bovidae is characterized by explosive radiation events that took place approximately 18-20 million years ago (MYA) according to fossil record (Vrba 1995). During the evolution of the bovids, over 137 extant species have diverged, making this family one of the most specious groups of mammals (Grubb 1993). Classification methods using morphology alone have divided Bovidae into six subfamilies and 12 different tribes (Ansell 1972, Gentry 1992). Global change during the Late Miocene and Early Pliocene created warmer temperatures and ample rainfall for savanna vegetation to dominate the African landscape (Cerling et al. 1992). This change sparked the major bovid radiation events within the subfamily Bovinae, including the emergence of the tribe Alcelaphini (Vrba 1979).

The Alcelaphini tribe arose in South Africa and spread throughout the African continent (Vrba 1995). This tribe comprises the following extant species: tsessebe (*Damaliscus lunatus*), hirola (*Beatragus hunteri*), red hartebeest (*Alcelaphus buselaphus*), Lichtenstein's hartebeest (*Alcelaphus lichtensteini*), black wildebeest (*Connochaetes gnou*), blue wildebeest (*Connochaetes taurinus*) and the blesbok/bontebok (*Damaliscus pygargus*). Genera of Alcelaphini have been regarded as being quite exceptional by way of sharing distinctive characteristics such as: horned females, specialized limb characteristics, extensive internal sinuses, short braincase and evolutionary advanced teeth (Gentry 1992). The evolutionary relationship of the tribe members supports a basal placement of *Connochaetes* to the sister taxa of *Damaliscus* and *Alcelaphus* (Matthee and Robinson 1999a) (Figure 1).

Figure 1. Evolution of the tribe Alcelaphini adapted from Matthee and Robinson 1999. The phylogeny was reconstructed using cytochrome b sequences by three methods of analysis (maximum parsimony, minimum evolution and maximum likelihood). All nodes are well supported by each method (Bootstrap value range 75 - 100%)



The fossil evidence places the first appearance of Alcelaphini at approximately 5 MYA (Vrba 1995) while mitochondrial DNA data supports an earlier emergence at 10 MYA (Hassainin and Douzery 1999) (Figure 2). The present alcelaphini species are contemporaneous and have arisen between 0.5 – 1.5 MYA according to the fossil record (Vrba 1979). Mitochondrial data supports the fossil dates for the most part, but has revealed extinction of some lineages of species (Arctander et al. 1999). Within this epoch, bovids underwent another macroevolution due to the shifts in temperature and rainfall throughout glacial and inter-glacial periods (Vrba 1979). Many warm regions of Africa with sufficient rainfall became refuges for bovids. These ecological fluctuations shaped the speciation events of some bovid species and lineages while at the same time caused the extinction of others.

Taxonomy and Subspecies Classification

Fossil evidence has revealed that *D. pygargus* had a continuous distribution covering the southwest cape region in South Africa to the southern area of Zimbabwe. However, over geologic time the species was split into two groups by climatic and habitat changes and have remained allopatric (Skinner and Smithers 1990) (Figure 3). This isolation has allowed for morphological and behavioral differences to arise in each group. Subspecies recognition of the bontebok (*D. p. pygargus*) and the blesbok (*D. p. phillipsi*) was based upon these differing characteristics. The common names given to each subspecies by the early settlers have become entrenched over 300 years and have been retained to represent each subspecies.

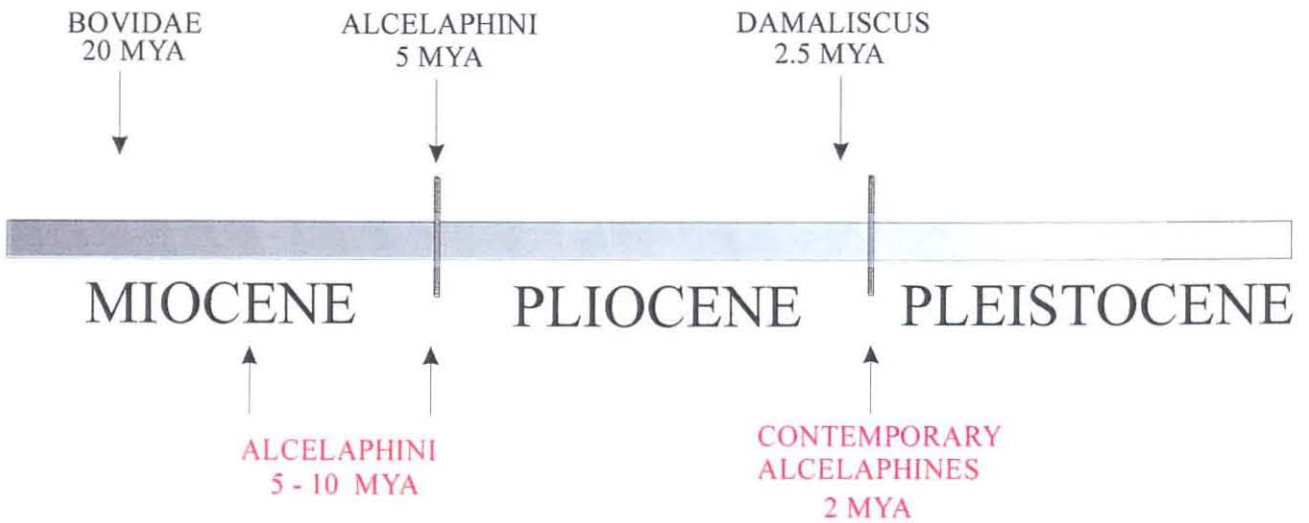


Figure 2. Geologic timescale of the major radiations of Bovidae leading to the emergence of *Damaliscus*. The fossil record dates are illustrated above the time line and are featured in black text. Molecular data place the emergence of Alcelaphini within the late Miocene - early Pliocene and the radiation of all contemporaneous alcelaphines during the Pleistocene.



Figure 3. Historical distribution of *Damaliscus pygargus*.

The scientific names of the bontebok and blesbok have changed several times over the last few hundred years due to the confusion over nomenclature codes (Table 1). In 1766, Pallas described a bontebok specimen as *Antilope dorcas* in his Miscellanea Zoologica. Later he realized that he had reserved that name for another species and subsequently changed the species name to *pygargus* in 1767 in his revised Spicilegia Zoologica. The classification became even more confusing when the genus name was reviewed by Scatler and Thomas (1894-1900) and later revised by Harper (1940). The classification was changed to the combination of *Damaliscus* and *dorcas*. Finally, the 1985 Code of Zoological Nomenclature (Article 59b) stated that a secondary homonym was invalid if changed before 1961. This code invalidates the *dorcas* homonym and supports the use of *pygargus* as species name (Rookmaaker 1991). Many researchers still continue to use the incorrect scientific name (*D. dorcas*) for the bontebok and blesbok antelope. The taxonomic description of this antelope species should further on be referred to as *D. pygargus* (Pallas, 1767).

Description of Physical and Behavioral Traits

Blesbok and bontebok are the smallest antelope of the Alcelaphini tribe (Estes 1991). The antelope were classified as separate subspecies based upon morphological differences and geographic isolation. Historically, bontebok were restricted to the coastal plains of the Western Cape region while blesbok roamed the grasslands of the Eastern Cape, Free State, Gauteng and Mpumalanga (former Transvaal). The subspecies have distinct color characteristics including coat color, body markings and facial blaze (Bigalke 1955) (Figure 4). The markings of the bontebok are quite striking having a dark,

Table 1. Nomenclature of *D. pygargus*

| | Taxonomy |
|--------------------------|--|
| Family | Bovidae |
| Subfamily | Hippotraginae |
| Tribe | Alcelaphini |
| Genus/Species | <i>Antilope dorcas</i> (Pallas 1766) |
| Species (revised) | <i>A. pygargus</i> (Pallas 1767) |
| Genus/Species (reviewed) | <i>A. pygargus</i> (Sclater & Thomas, 1894) |
| Genus/Species (revised) | <i>Damaliscus dorcas</i> (Harper 1940) |
| Bontebok subspecies | <i>D. d. dorcas</i> (Pallas, 1767) |
| Blesbok subspecies | <i>D. d. phillipsi</i> (Harper, 1940) |
| Reclassification | (Rookmaaker, 1991) |
| Genus/Species | <i>Damaliscus pygargus</i> (Pallas, 1767) |
| Bontebok subspecies | <i>D. p. pygargus</i> |
| Blesbok subspecies | <i>D. p. phillipsi</i> |



(J. van der Walt 1998)



(D.Lynch 1998)

Figure 4. The blesbok (*D.p. phillipsi*) is shown on the right in its grassland habitat while the bontebok (*D.p. pygargus*) is figured in renoster shrubland on the left. The horn and body color of blesbok are normally darker than that of bontebok. Each subspecies is distinguished by a white face blaze which is continuous in bontebok and disrupted in the blesbok by a horizontal brown band. The white rump patch is characteristic of bontebok.

purple sheen to the coat, large white rump patch surrounding the tail, and white lower legs and belly. Bontebok have a white facial blaze, which runs continuously from the base of the horns to the nose. The color of the blesbok is lighter brown with a light tan rump patch and tawny legs and belly. Blesbok are also distinguished by a white facial blaze that is divided by a light brown band between the eyes.

The social structure of the bontebok consists of small nursery herds (females and calves), territorial males and large herds of bachelor males throughout all seasons. The bachelor and nursery herds rove between the park boundaries in search of short grass while reproductive males defend territories and display to mature females (David 1973). The social structure of the blesbok differs somewhat from that of the bontebok. Reproductive males strongly defend territories during the breeding season, like the blesbok; however, all territories are given up after the rut in the winter season (June – August). Large aggregations of antelope of every age and sex are formed to increase individual likelihood of grazing during the dry months. All herds re-group in November after calving begins and territorial males re-establish territories for the next breeding season (Lynch 1971).

Early Genetic Studies

Although ecological and behavioral data exists for *D. pygargus*, little genetic data is available for this antelope. An early genetic study of blesbok using protein markers (transferrin, hemoglobin, amylase, albumin and carbonic anhydrase) revealed no variation for these blood factors (Osterhoff et al. 1972). The same study did, however, report slight variations in blood serum of blesbok using antisera to goat red cell factors. A second study screened the mitochondrial genome for variation using restriction fragment length

polymorphism (Essop et al. 1991). The structure and mode of mitochondrial DNA (mtDNA) evolution provides a finer measure of genetic variation than protein markers below the species level (Avisé 1994). The restriction maps revealed genetic difference between the bontebok and blesbok and a calculated sequence divergence of 0.47% (Essop et al. 1991). This study also estimated an approximate time of divergence between blesbok and bontebok at 250,000 years based upon the mean rate of mtDNA divergence of 2% per million years (Wilson et al. 1985). Lastly, G and C banded karyotypes ($2n = 38$) of both subspecies displayed complete homology (Kumamoto et al. 1996).

Bontebok and Blesbok Conservation

The former distribution of the bontebok covered the coastal sandveld of the southwestern Cape region from the settlements of Caledon and Mossel Bay (van der Merwe 1968). An array of geographic barriers, including the Bot River in the west, the Langeberg and Zonderend mountain ranges in the north and the Atlantic and Indian oceans of the south restricted movement of bontebok from the narrow confines of this region (Figure 5). Human settlement within the western Cape began in 1652 and occupied the most fertile land for agriculture. This encroachment forced the bontebok herds into the less suitable grasslands for grazing. Large herds of bontebok and other antelope were still sighted by the early Cape naturalists in the late 1600's and 1700's, then drastically declined by the early 1800's (Skead 1980). Intensive human encroachment and strong hunting pressure attributed to the near extinction of the endemic South African bontebok.

Fortunately, several landowners within the area had the insight and initiative to protect herds of bontebok on their farms, which in turn, led to the recovery of this

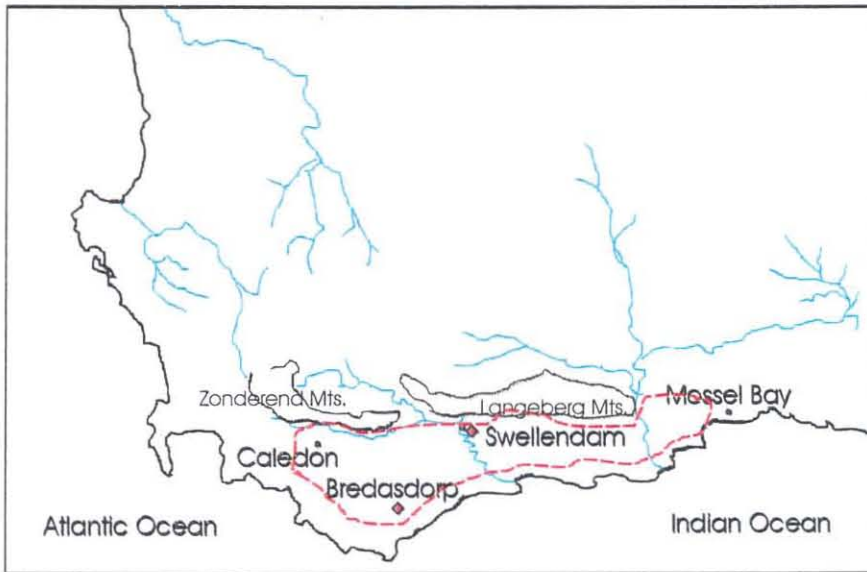


Figure 5. The historic distribution of bontebok is shown by the red outline. Due to the drastic reduction in population size, the first Bontebok National Park (BNP) was proclaimed in Bredasdorp in 1931. Surviving animals from the original herd were translocated in 1960 to the present BNP located in Swellendam.

antelope. In 1837, a small herd of 27 bontebok was protected on a farm owned by the van der Byl family within the Bredasdorp area. The herd number increased slowly at two animals per year but tended to decrease during seasons of drought. By the early 1900's, the total number of protected bontebok increased to approximately 250 individuals on the combined area of the van der Byl and Albertyn farms (Skead 1980).

In 1931, the first Bontebok National Park was proclaimed in Bredasdorp in order to protect the remaining bontebok from further hunting pressure. The park was stocked with 17 bontebok from the van der Byl and Albertyn farms (N. Fairall–National Parks Board). Within three decades, this herd increased ($n = 180$) well beyond the carrying capacity of the small reserve. The only remedy at the time was to graze bontebok on artificial pastures that were deficient in copper (Barnard and van der Walt 1961). The deficiency presented in the forms of swayback and ataxia in the antelope. Animals also suffered from massive worm infestations of conical fluke (*Paramphistomum* sp.), lung worm (*Protostrongylus* sp.), wireworm (*Haemonchus* sp.), brown stomach worm (*Ostertagia* sp.) and bankruptworm (*Tirchostrongylus* sp.) which aggravated the swayback condition. A large mortality (50%) resulted within the herd from the parasitic infection, poor grazing conditions and related syndromes. A decision was made to translocate antelope from the park to a suitable habitat farther north in Swellendam. The new Bontebok National Park was established in 1960 with 61 bontebok that survived the translocation event. The estimated 2,500 - 3,000 bontebok today are derived from this founding population. Bontebok are still considered a rare antelope species and are listed as vulnerable in Appendix II of the International Trade in Endangered Species Red List (World Conservation Union, 1993).

The blesbok antelope were also persecuted by the early settlers for their hides and were reported as having been slaughtered by the thousands after the Great Trek in 1836

(Lynch 1971). The naturalist H. A. Bryden (1893) wrote “The blesbok which not long since scoured the plains of Lower Bechuanaland (Northwest Province SA-Botswana), the Transvaal (Guateng and Mpumalanga) and Free State in the countless thousands, is now seldom seen”. The extermination of blesbok populations persisted until 1899 when the trade in skins was brought to an end by the Boer War. After the war, the majority of the open grassland regions were taken over by settlers forcing the remaining populations to exist only on fenced farms. Their distribution was then artificially expanded by translocation events to the Cape region and Natal. By 1962, the population size of the South African blesbok totaled approximately 42,000 (Kettlitz 1967). Blesbok are still a popular game antelope today, however, the hunting is now confined to private lands and national hunting concessions. The population size of blesbok is currently 120,000 (David 1998).

The subspecies had been separated by over 320 km and remained allopatric until recent times. Many herds have been translocated out of their natural ranges to stock private farms and reserves. The blesbok and bontebok antelope readily hybridize given their close evolutionary relationship. Animals resulting from these hybridizations were also translocated throughout South Africa (Allardice and Gaigher 1979). The genetic admixture of the subspecies threatens the genetic purity of the bontebok. Hybrid animals look very similar to blesbok but are larger in body size (N. Fairall per com.). The blesbok are normally darker in color; however, pelage can become very pale in color if copper is deficient in their diet (Penrith et al. 1996). The white markings can also be used to identify subspecies such as the amount of white on the legs and the shape of the facial blaze. Fabricius et al. (1989) used a discriminant function analysis of rump patch measurements to differentiate between the pure subspecies and hybrid animals. They

reported that the hybrid scores fell between the continuum of the pure subspecies. Parks Board officers have used this method to verify purity of bontebok and blesbok herds.

2. Conservation of Genetic Diversity

Conservation Genetics

The endangered status of a species largely results from drastic population reduction caused by habitat destruction, over-hunting, disease outbreaks or environmental changes. Massive global population declines of vertebrate species have been noted as early as the 18th century, but still today, the numbers of endangered and extinct species have escalated to a tragic figure. A new scientific discipline-conservation genetics-has surfaced from these catastrophic events. This science is used to conserve biodiversity by applying the theories of molecular evolution and population genetics and the new methods of biotechnology (Avice 1989, Moritz 1994a, O'Brien 1994a, Hedrick 1999). The genetic data created by molecular techniques can provide traces of historical events as well as the present status of the species (O'Brien 1994b). Genetic findings, together with ecological, behavioral, demographic, and clinical data, can be used to design management plans for the conservation of the species.

Genetic diversity has been slated as an important level of biodiversity demanding conservation (McNeely et al. 1990). This recognition aims to preserve the existing genetic variation of the species for future evolutionary change. Having a substantial amount of variation, the species is better able to adapt to new ecological changes and disease challenges (Fisher 1930, Frankham 1996). This evolutionary potential has been largely theoretical until recent studies have provided empirical data that supports the relationship between genetic variation and fitness (Frankham et al. 1999).

Inbreeding depression

In natural populations, severe population contractions often lead to inbreeding within a population. Mating between close relatives causes the decline of the value of a trait (Wright 1931) and loss of fitness. Although, inbreeding depression is slowly becoming accepted as an important factor on species survival, the impact is still argued against by some (Caro and Laurenson 1994). Because the physical symptoms of inbreeding depression are difficult to observe in the wild, the threat has often been ignored. However, Cmokrak and Roff (1999) have thoroughly reviewed the ill effects of inbreeding in wild populations. The consequences of inbreeding depression are the following: increased incidence of hereditary disorders, loss of fitness and increased risk of disease susceptibility.

Hereditary disorders

The genetic defects are caused by inheritance of deleterious alleles that may have become fixed in the population (Charlesworth & Charlesworth 1987, Lande 1998). This kind of inbreeding depression results from inheritance of lethal alleles at one single locus or a few loci. The amount of lethal alleles found in a population was termed as the genetic load by Crow in 1930. Laikre (1999) summarized current observations of inherited diseases found in captive carnivore species, which include hereditary blindness, cryptorchidism, albinism, skeletal defects, and congenital heart anomalies. Similar conditions of heart defects, cryptorchidism and vertebrate deformity (kinked tail) have been found in the relic population of Florida panther (Roelke et al. 1993).

Fitness traits

Inbreeding is also associated with loss of individual fitness and population viability. The traits affected by inbreeding depression are related to reproductive fitness, fecundity, and juvenile survival (Wright 1977). These consequences of inbreeding depression had been documented in humans (Morton et al. 1955) as well as domestic (Wright 1922) and laboratory animals (Bowman and Falconer 1960). But the threat to exotic animals was only made evident in 1979 by a seminal paper published on inbred captive ungulates by Ralls and colleagues. They reported on a high degree of juvenile mortality in captive inbred ungulate species and suggested that the same phenomenon may occur in the wild. Pedigrees of captive exotics have also been used to estimate inbreeding coefficients and the “costs” of inbreeding (juvenile mortality) in each population (Ralls et al. 1988). Again, this work demonstrated the negative effects of inbreeding and highlighted the possibility that it may pose a severe threat to natural populations.

Sperm quality is another reproductive trait that can be negatively affected by inbreeding depression. The morphology, mobility, viability and concentration of sperm are important factors for successful fertilization to occur (Drobnis and Overstreet 1992). These sperm traits, and other characteristics, have been intensely studied in wild felid species that have undergone drastic population reductions. The African cheetah (*Acinonyx jubatus*), the Ngorongoro lion (*Leo leo*), and the Asian lion (*Leo persica*) all demonstrate reduced male reproductive fitness due to poor sperm quality (Wildt et al. 1983, 1987). Two studies of captive gazelle species (*Gazella sp.*) confirmed that individual inbreeding coefficients are related to percentage of normal sperm and length of sperm mid-piece (Roldan et al. 1999, Gomendio et al. 2000). These reports provide

strong evidence for the high reproductive costs associated with inbreeding depression in ungulates.

Fluctuating Asymmetry

Fluctuating asymmetry (FA) is defined as the deviation of a morphological character (e.g., fin length, horn width) from normal bilateral symmetry (Van Valen 1962). The degree of asymmetry has been hypothesized to intensify due to genetic factors (inbreeding, hybridization) or severe environmental conditions (pollution, habitat changes). These factors may alter the normal development of the organism and cause an increase in fluctuating asymmetry (Palmer and Strobeck 1986). It has been suggested that levels of FA have been negatively correlated to levels of heterozygosity (Mitton 1993). Moreover, FA has been strongly linked with genetic stress (inbreeding) and ejaculate quality in gazelles (Roldan et al. 1999, Gomendio et al. 2000).

Predisposition to disease

High genetic diversity is thought to promote greater fitness as well as higher disease resistance in a population (Hughes 1994). The consequences of infectious disease on a host species depend on many factors including “ecological and epidemiological conditions associated with the outbreak” (Murry et al. 1999). Moreover, the severity of the disease may be affected by factors other than inbreeding, such as the nutritional index, pathogen load and stress levels of the animal (Ullrey 1993). The relationship between genetic diversity and disease susceptibility is often difficult to prove in natural populations given the complexity of disease manifestation. However, a recent study has

related individual heterozygosity levels with disease resistance and survival within a wild population of Soay sheep (Coltman et al. 1999).

Genetic Introgression

Hybridization between closely related species or subspecies is one of the natural processes of speciation (O'Brien and Mayr 1991). This natural occurrence may develop from changes in ecology, which unite formally allopatric species ranges. It has been postulated that the red wolf (*Canis rufus*) originated by this process when the grey wolf (*Canis lupus*) and coyote (*Canis latrans*) hybridized during the late Pleistocene (Reich et al. 1999). However, most hybridization events are caused by un-natural or man-induced situations. In these cases, exotic species or subspecies are introduced to an area that is inhabited by native taxa. The subsequent mating events ultimately contaminate the genetic purity of the native species. For endemic species and subspecies, genetic introgressions pose a great risk to their survival as distinct evolutionary lineages. Furthermore, failure to protect these lineages from extinction represents a loss in biodiversity.

Illegal translocations of vertebrate species for economical gain have created numerous conservation problems in southern Africa. Antelope species, in particular, are moved out of their natural range to game farms for trophy hunting. Hybridization events are common between antelope subspecies including the bontebok and blesbok (Allardice and Gaigher 1979), and also the black-faced impala (*Aepyceros melampus petersi*) and common impala (*A. m. melampus*) (Green and Rothstein 1997). The severity of the problem is also evident in the hybridization between species such as black and blue

wildebeest (Fabricius et al. 1988) and the red hartebeest and tsessebe (Robinson et al. 1991).

Hybridization between closely related breeds, races or subspecies may result in two very different manifestations of heterosis. The fitness costs or consequences of the hybrids may vary greatly among species (Charlesworth and Charlesworth 1987). In one case, a negative effect of hybridization may cause the reduction of fitness of the offspring in outbreeding depression. That is, loss of fitness will occur if the two parental groups have evolved local adaptations such as mating behaviors, feeding strategies and disease resistance (Templeton 1987). In this regard, hybridizations may result in unfit progeny that are not successful in the wild (Hatfield and Schluter 1999).

In another scenario, hybridization can result in positive heterosis or “hybrid vigor”. This cross-mating between groups is thought to cause the purging of deleterious alleles at homozygous loci and the subsequent increase heterozygosity levels (Crow 1948). Therefore, the effects of inbreeding will be diminished and the progeny will inherit higher fitness (Thornhill 1993).

2. Evolutionary Units for Conservation

The Biological Species Concept (BSC) recognizes that the genetic diversity of a species can be subdivided among separated populations into subspecies (Mayr 1963). Intraspecies classification, or taxonomy below the species level, has historically been based upon phenotypic differences and geographic distribution. Morphological characteristics such as pelage, stripe pattern and body size have been used to describe subspecies of mammals. In most cases, geographic distance or physical boundaries

prevent contact and gene flow among subspecies. The temporal and spatial distribution of the subspecies allows for genetic differences to arise (O'Brien and Mayr 1991).

These genetic differences are being utilized to describe evolutionary lineages for conservation (Ryder 1986). This concept emerged out of necessity for genetic diversity to be included in the criteria used for conservation management. An evolutionary significant unit (ESU) was originally defined as any population having a distinct evolutionary history, adaptive differences and reproductive isolation (Ryder 1986, Waples 1991). Through genetic analysis, the taxa that are significantly divergent can be recognized as an ESU and prioritized for conservation. Further refinement of the concept has incorporated reciprocal monophyly for mtDNA sequences and divergence of nuclear gene frequencies to identify units for conservation (Moritz 1994a). Populations that display allele frequency differences are defined as management units (MU). These groups are not phylogenetically distinct, but they do however, show structure by way of frequency distribution that is determined by breeding units within the population (Moritz 1994b).

Since the inception of the ESU concept, biologists have argued that many populations have been labeled as distinct units based solely on genetic data and have failed to include ecological perspectives (Taylor and Dizon 1999, Crandall et al. 2000). They caution the abuse of ESU terminology that limits the definition of conservation units to only phylogenetically distinct taxa. Crandall and colleagues (2000) also highlight that such treatment of taxa may prevent the maintenance of evolutionary potential by restricting gene flow and adaptation.

4. Measurement of Genetic Diversity and Substructure

The recent advances in genetic technology have made it possible to address specific questions concerning patterns of genetic diversity found within natural populations (Sunnucks 2000). The highly variable molecular markers described in the following chapters have been found to be highly informative in the examination of the genetic dynamics of populations.

5. Aims of Study

Hypothesis

Damaliscus pygargus diverged into two genetically distinct subspecies through geographic isolation over a long period of time.

Objectives

1. Evaluate the extent and character of genetic differentiation between the bontebok and blesbok in order to:
 - a. Validate or contest subspecific classification.
 - b. Infer past demographic events for each subspecies.
 - c. Detect hybridization events between the subspecies by genetic screening methods.