Major Histocompatibility Complex Diversity in an Urban Chacma Baboon (*Papio ursinus*) Population: Implications for Conservation

JENNECA M. MCCARTER
Supervisor: Dr. Jacqueline Bishop
Department of Zoology, University of Cape Town

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Abstract

Since the 15th century, human activity has altered and degraded nearly half of the available land of the Cape Peninsula, South Africa; this has resulted in significant restriction and fragmentation of the historic geographic range of the peninsula's Chacma baboon (*Papio* ursinus) population. Extensive contemporary urban sprawl today restricts sub-adult male baboons from dispersing from natal groups to and from troops elsewhere in the Western Cape, South Africa resulting in reduced gene flow across the landscape together with a loss of neutral genetic within the peninsula's baboons. This loss of genetic variation, coupled with close proximity to humans and the environmental pressures of existing in an urban landscape, raise concerns about the potential for pathogen mediated population declines for baboons and the emergence of infectious diseases. In this study genetic variation at gene loci responsible for the adaptive immune response is examined and discussed in the context of a conservation approach that includes translocation as a potential management tool for introducing novel genetic variation to Chacma baboons of the Cape Peninsula In place of extensive and costly disease screening, data from the second exon of the major histocompatibility complex (MHC) *DQA* locus was examined as a proxy for the pathogen environment of troops sampled across the Western Cape. Results indicated that Chacma baboons exhibit high levels of allelic diversity at the MHC-*DQA*1 locus. Sequences described in this study represent two allelic lineages at the *DQA*1 locus that have evolved under strong selection for functional diversity in the presence of pathogens. One allele (Paur_*DQA*1*01) was recovered at high frequencies (43-92%) at all ten sample sites, and alleles representing the two distinct allelic lineages were found among baboon populations both on and off the peninsula. Data from this study augments the findings of previous work on mitochondrial genetic variation and preliminary pathogen screenings; these studies together provide support for a management regime that should consider the translocation of animals as an effective tool to ensure the persistence of an evolutionarily fit population of Chacma baboons on the Cape Peninsula.

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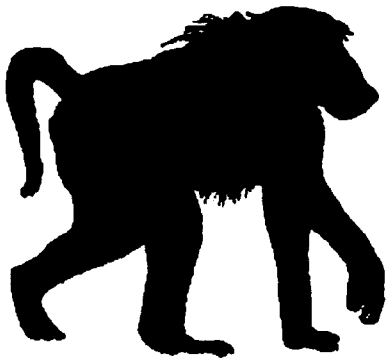

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Chapter 1

Literature Review

Introduction

The decline in biodiversity worldwide is attributable to a number of human-induced factors, principally those activities linked to habitat loss and fragmentation (Wilson 1992; Pimm et al. 1995; Pimm & Raven 2000). As more natural habitat is fragmented and destroyed, wildlife populations become increasingly isolated. This ongoing process disrupts dispersal behaviors and thus gene flow across the landscape, ultimately increasing the risk of a loss of genetic variation via genetic drift and inbreeding (Templeton et al. 1990; Keller & Waller 2002; Frankham et al. 2002; Reed & Frankham 2003). Loss of genetic variation can lead to decreased fitness through reduced infant survival and reproductive success, as well as a decreased ability to adapt to environmental changes (Lande 1988; Lacy 1997; Frankham & Ralls 1998) and increasingly, studies also demonstrate that genetic variation is crucial for protecting wildlife populations against pathogens and disease epidemics (Keller & Waller 2002; Spielman et al. 2004). This suggests that isolated populations with limited dispersal may be at a heightened risk of population decline due to greater susceptibility to infectious disease (Lyles & Dobson 1993; McCallum & Dobson 1995). In an effort to assess the level of risk wildlife populations are facing, conservation biologists often turn to genetic information as a means to measure levels of individual and population level fitness.

While many genetic studies concerning fitness of wild animals utilize neutral markers such as mitochondrial DNA to estimate the remaining levels of genetic diversity present in a population, variation at these loci cannot provide information about fitness and the selective processes acting

on species and their pathogen environment or the capability of species to adapt to future change (Meyers & Bull 2002; van Tienderen et al. 2002; Sommer 2005). These are important issues for conservation biologists, as the ability to adapt to environmental changes is crucial for the persistence of evolutionarily fit populations (Sommer 2005). Genes under selection, such as those of the highly variable major histocompatibility complex (MHC) of the vertebrate immune systems, reflect adaptive processes within and between populations (Sommer 2005). Accordingly, data on MHC variation can inform conservation biologists about extant fitness of a population, as well as the potential for adaptation in the face of rapidly changing environments.

The Major Histocompatibility Complex

All vertebrate immune systems comprise both innate and adaptive immune responses. The innate immune system is believed to have predated the adaptive system, and is the first line of immune response where molecules recognize certain pathogen-associated molecular patterns (Medzhitov & Janeway Jr. 1997; Kindt et al. 2006). If pathogens evade the innate response the second line of defense, adaptive immune response, takes over. The adaptive immune response creates an immunological memory after initial introduction to a pathogen so that there is an enhanced response for any subsequent encounter (Kindt et al. 2006). Central to adaptive immune response is a cluster of genes, the major histocompatibility complex (MHC), whose gene products assist with self/non-self discrimination and recognition of foreign antigens (Kindt et al. 2006). For example, loci of the MHC play a major part in determining if transplanted tissues are rejected as foreign (histoincompatible) or accepted as self (histocompatible), and play a key role in the development of cell-mediated and humoral immune responses (Kindt et al. 2006).

Structure and Function of the Major Histocompatibility Complex

Genes of the MHC encode receptor glycoproteins expressed on the surface of cells; these receptors process and present foreign peptides obtained from parasites and pathogens to specialist immune cells (Sommer 2005; Piertney & Oliver 2006), triggering the adaptive immune response in the host by activating key immunological players such as B cells, macrophages, T-cells, and cytotoxic T-cells (Penn & Potts 1999).

Classical MHC genes are subdivided into two major groups, referred to as class I and class II genes (Hughes & Yeager 1998; Sommer 2005). Based on the well-studied human MHC gene region, the loci of these genes have been labeled human leukocyte antigen (HLA) *-A, -B, -C* (class I) and *-DP, -DR, and -DQ* (class II) (de Groot et al. 2002). Class I molecules are structured by an α protein chain which is associated with a β microglobulin peptide produced by a gene from outside the MHC (Nei et al. 1997; Knapp 2002). After binding peptides from sources such as viruses and cancer infected cells, class I molecules present these endogenously derived antigens to CD8+ cytotoxic T-cells which then proceed to lyse the infected cell (de Groot et al. 2002; Sommer 2005; Piertney & Oliver 2006).

Class II molecules are heterodimers composed of non-covalently associated α and β chains, which are encoded by two separate genes (Hughes & Nei 1990; Piertney & Oliver 2006). Class II genes encode for glycoproteins on antigen presenting cells such as dendritic cells, macrophages, and lymphocytes (Piertney & Oliver 2006). In contrast to class I receptors, molecules derived from class II genes are engaged in identifying extracellular pathogens such as bacteria and parasites (Sommer 2005). These exogenously derived antigens are presented to CD4+ T-helper cells (Piertney & Oliver 2006; Averdam et al. 2011) which triggers the T-helper

cells to release cytokines in turn activating immune responses, such as the production of antibodies (Hughes & Yeager 1998).

All MHC receptors are anchored to the cell surface by an immunoglobulin 'stalk' and possess a receptor 'basket', referred to as either the peptide binding region (PBR) or antigen binding site (ABS), which is responsible for recognition and presentation of antigens to the cells of the immune system (Figure 1.1) (Sommer 2005; Piertney & Oliver 2006).

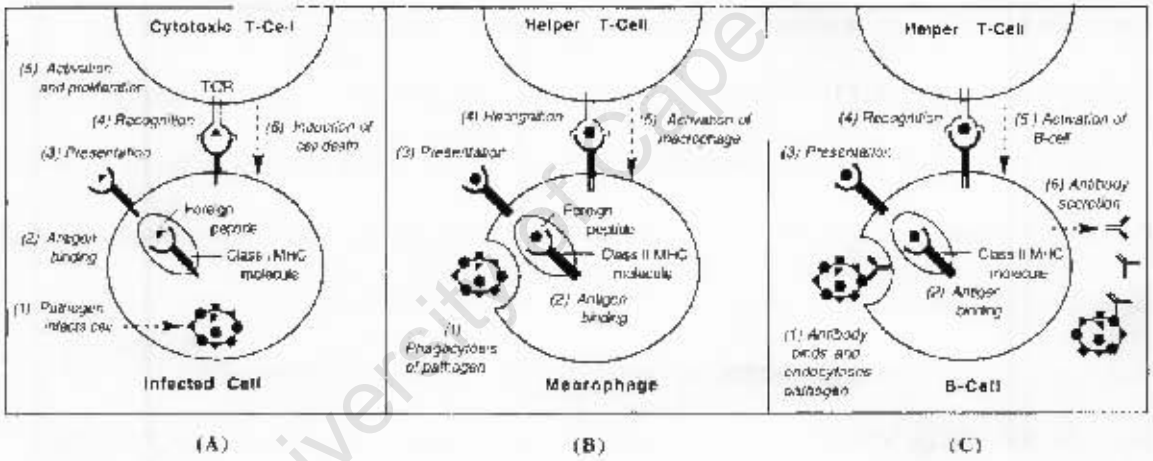


Figure 1.1: Adaptive immune response assisted by MHC. (A) MHC class I receptors present endogenously derived antigens to cytotoxic T-cells. (B and C) MHC class II receptors are expressed on antigen presenting cells and present exogenously derived antigens to helper T-cells. (Taken from Penn & Potts 1999)

The antigen binding site of a single MHC molecule is able to bind numerous antigens which possess common amino acids at particular anchor positions (Altuvia & Margalit 2004). Antigen binding sites exhibit high levels of variation both in the number of alleles and the degree of variation between alleles that they are encoded by (Hughes & Yeager 1998; Sommer 2005). Indeed, the MHC comprises the most polymorphic genes currently known in vertebrates, with

multiple copies of loci and numerous alleles at each locus (Hedrick 1994; Hughes & Hughes 1995; Sommer 2005; Piertney & Oliver 2006). MHC receptors each possess their own set of binding properties, resulting in different peptides being bound by different receptors (Knapp 2005a). MHC molecule variability is associated with diversity in T-lymphocyte receptors, and in this way determines a host's resistance or susceptibility to different pathogens (Hedrick et al. 2001a; Hedrick et al. 2001b; de Groot et al. 2002; Sommer 2005). Individuals with a suite of highly variable MHC alleles are therefore able to recognize a greater diversity of antigens and theoretically should be more adept at fighting disease than less variable individuals (Piertney & Oliver 2006). Increasingly, empirical evidence supports the theory that variability in amino acid composition at the ABS alters a host's resistance to specific pathogens. For example, in Malagasy mouse lemurs (*Microcebus murinus*) three MHC alleles found to be strongly correlated with gastrointestinal parasite susceptibility exhibit unique changes in amino acids at the antigen binding site and two of these (*Mimu-DRB*6* and *Mimu-DRB*10*) are associated with significantly lower parasite loads in the mouse lemurs (Schad et al. 2005). MHC diversity is therefore predicted to increase fitness at the population level, as individual variation should lower the probability of pathogens sweeping through a population (de Groot et al. 2002). For example, present day common Chimpanzees (*Pan troglodytes*) are thought to have lost certain MHC alleles during the course of evolution. Chimpanzees exhibit a reduced MHC class I allele repertoire that may have been due to an ancient selective sweep caused by an SIVcpz or a related ancestral retrovirus pandemic in the ancestral Chimpanzee population (de Groot et al. 2002).

Selection Mechanisms on the MHC

The highly polymorphic nature of certain loci within the MHC has led to extensive research investigating the mechanisms maintaining this diversity. A variety of selective pressures have been suggested as mechanisms, including pathogen driven selection, sexual selection, and maternal-fetal interactions (Potts et al. 1991; Penn & Potts 1999). Doherty and Zinkernagel (1975) proposed the first major hypothesis explaining MHC polymorphism based on pathogen driven selection (Hughes & Yeager 1998). Their hypothesis, coined the 'overdominance hypothesis' or 'heterozygote advantage hypothesis', stated that polymorphism could be explained due to the advantageous nature of being a heterozygote at MHC loci (Doherty & Zinkernagel 1975; Hughes & Yeager 1998). They proposed that heterozygotes would recognize a broader spectrum of pathogens than homozygotes, thus potentially conferring a greater level of fitness (Doherty & Zinkernagel 1975; Hughes & Yeager 1998; Sommer 2005). To test the heterozygote advantage hypothesis more thoroughly, Hughes and Nei (1988) examined rates of non-synonymous (d_n) to synonymous nucleotide substitutions per site for class I genes expecting to find some evidence of balancing selection on the genes (Hughes & Yeager 1998). At neutral genes the rate of synonymous nucleotide substitutions (d_s) generally exceeds that of non-synonymous substitutions (d_n) (Kimura 1983). This is due to the fact that non-synonymous substitutions alter the amino acid composition of a gene product and have the potential to be deleterious (Hughes & Nei 1989; Sommer 2005). Hughes and Nei (1988) found that MHC loci follow the expected synonymous to non-synonymous substitution ratio ($d_s > d_n$) of neutral evolution, except at antigen binding sites. While the antigen binding sites indeed displayed a significantly higher rate of non-synonymous (d_n) to synonymous (d_s) substitution (Hughes & Nei 1988; Hughes & Nei 1989) synonymous substitution rates in the non-ABS sites were found to be

uniform across gene regions, indicating the high rates of non-synonymous substitutions in ABS sites could not be explained by overall heightened mutation rates alone (Brown et al.1993; Hughes & Yeager 1998). The findings instead suggested that nucleotide diversity in MHC genes may be the result of balancing selection (Hughes & Nei 1988; Hughes & Nei 1989; Bernatchez & Landry 2003). A few studies provide convincing evidence for heterozygote advantage. Most supporting data comes from MHC heterozygous laboratory mice who display quicker clearance of parasites (e.g. the trematode *Schistosoma mansoni,* Sher et al. 1984; and the nematode *Heligosomoides polygyrus,* Behnke & Wahid 1991*)* and less susceptibility to bacterial and viral infections (streptococcus induced lesions, Chen et al. 1992; *Salmonella and Lysteria,* Penn et al. 2002; *Salmonella enterica* and Theiler's virus, McClelland et al. 2003) than their homozygous counterparts. There is also support for this theory from wild populations; in Chinook salmon (*Oncorhynchus tsawytscha*) heterozygotes at MHC class IIB loci display higher survival rates than homozygotes when confronted with haematopoietic necrosis virus (Arkush et al. 2002) and in Gila topminnows (*Poeciliopsis o. occidentalis*) infected with an exotic fluke, MHC heterozygotes display a 15.5% higher survival rate than homozygotes (Hedrick et al. 2001a).

While it has been widely accepted that MHC polymorphisms are maintained by balancing selection, the mechanisms which drive this process are still heavily debated (Sommer 2005; Piertney & Oliver 2006). 'Frequency-dependent selection' is another pathogen driven model which proposes that rare alleles may confer an advantage at a certain frequency (Takhata & Nei 1990). Because pathogens are continuously adapting in order to infect the common genotypes in a population (Lively & Dybdhal 2000) rare alleles may have lower risk of infection, providing a selective advantage over common alleles (Harf & Sommer 2005). Eventually these rare alleles will increase in frequency through the population due to host advantage and become the new

common genotype, forcing the pathogens to alter their antigenicity. The co-evolutionary process between host immune defense and parasite efficiency causes fitness levels of different alleles to fluctuate over time, resulting in the maintenance of overall high levels of genetic diversity (Sommer 2005). Empirical studies have provided evidence of rare MHC alleles providing resistance to pathogens such as Epstein-Barr virus and hepatitis B in humans (Thursz et al. 1995; Decamposlima et al. 2003).

A growing body of evidence also suggests that sexual selection may be an important mechanism in the maintenance of MHC polymorphisms (Sommer 2005). The reproductive behavior of dis-assortative mating, where organisms choose mates with MHC genotypes different to their own, may increase the chance of offspring being heterozygote at MHC loci (Edwards & Hedrick 1998). This ensures that the offspring will be able to recognize a greater array of pathogens than either parent and thus have increased fitness. Mating preference based on MHC genotype has been extensively studied and supported in laboratory mice (Yamakazi et al. 1976; Egrid & Brown 1989) as well as in populations of wild mice (Potts et al. 1991). To a lesser degree, the pattern of dis-assortative mating due to MHC genotype has also been detected in human populations (Jordan & Bruford 1998). Many studies provide evidence for lower rates of homozygous MHC loci than would be expected under random mating scenarios, but whether this nonrandom mating is attributable to MHC genotype or other factors remains a puzzle (Alberts & Ober 1993; Kostyu et al. 1993; Markow et al. 1993; Havlicek & Roberts 2009). It is also important to note that a number studies have provided evidence for mate choice independent of MHC genotype, e.g. great reed warblers (*Acrocephalus arundinaceus)* (Westerdahl 2004), rhesus macaques (*Macaca mulatta)* (Sauermann et al. 2001), and Soay sheep (*Ovis aries)* (Paterson &

Pemberton 1997). What can be concluded from MHC sexual selection studies across taxa is that mate choice may be based on anything from overall diversity to genotypic dissimilarity, or specific alleles (Piertney & Oliver 2006). The way in which individuals asses MHC types in one another has been correlated to olfaction (Brown & Eklund 1994). MHC genes have the ability to alter the concentration of volatile acids in urine or sweat which produce odor, perhaps providing a cue of MHC genotype or relatedness among individuals (Wedekind et al. 1995; Wedekind & Furi 1997; Beauchamp & Yamazaki 2003; Piertney & Oliver 2006). The ability to assess MHC types among organisms has also been correlated to secondary sexual characteristics (Piertney & Oliver 2006). For example, white tailed deer (*Odocoileus virginianus*) present MHC *DRB* genotypes which are associated with body size and rates of antler development. They also display a negative relationship between abundance of abomasal helminths and antler size, thus providing females with an indication of parasite resistance in potential mates (Ditchkoff et al. 2001).

MHC polymorphism is also thought to be influenced by maternal-fetal interactions (Hedrick & Thomson 1988). A fetus with diversity at MHC loci not shared with its' mother is thought to have a higher survival potential than a fetus who shares all diversity at MHC loci (Gill 1983). Evidence for this controversial hypothesis is found in the MHC loci of human couples with a history of spontaneous abortions, who are more likely to share diversity at MHC loci than control couples (Thomas et al. 1985). Maintenance of MHC polymorphism can thus be reinforced by maternal-fetal interaction, as it is selectively advantageous for the fetus to have multiple non-shared alleles (Hedrick & Thomson 1988; Clarke & Kirby 1966).

The unprecedented amount of diversity found in the MHC is not the result of a single evolutionary process, but instead appears to be the compound result of multiple selective pressures. While it seems clear that the major histocompatibility complex is under the influence of balancing selection at certain nucleotide sites, the exact mechanisms by which this selection occurs are still unclear (Piertney & Oliver 2006).

MHC diversity and conservation of natural populations

The availability of genetic information for wild populations has provided conservation biologists with a relatively accurate way to assess fitness and examine potential risks. Since diversity at MHC loci is clearly important for ongoing pathogen resistance, studies of this gene region serve as a good surrogate for understanding current fitness levels and the potential for host adaptation to future changes in their pathogen environment (Takahata & Nei 1990; Sommer 2005). This is of immense importance since pathogen-mediated population declines are predicted to increase due to the rise of anthropogenic manipulation of the natural environment (Smith et al. 2009). MHC data can also be utilized to inform conservation actions, such as the translocation of animals. One of the main arguments for translocation is to restore gene flow between once connected but now isolated populations in fragmented landscapes (Moritz 1999; Fischer & Lindenmayer 2000). The International Union for the Conservation of Nature (IUCN) requires that before animals are moved, detailed data on disease are acquired to ensure the individuals being moved have a high probability of survival in their new environment and won't introduce potentially devastating novel pathogens to both the receiving population and other native fauna (Soorae & Baker 2002). As extensive pathogen screening of wild populations is both logistically challenging and very expensive, MHC data can provide a more cost effective way to characterize

aspects of the pathogen history environment in which a population has evolved. In addition to using translocation to restore gene flow across fragmented landscapes, it can be used to supplement wildlife populations with individuals in order to bolster genetic diversity in inbred populations (Tallmon et al. 2004; Hedrick & Fredrickson 2010; Boyce et al. 2011). Theoretically, if individuals from one population display diversity at MHC loci similar to the target population, they have evolved in similar pathogen environments and movement of animals should carry lower risks (Sommer 2005; Piertney & Oliver 2006).

While the study of MHC genes can open up a whole new realm of information for conservation biologists, it is important to remember that the adaptive immune response mediated by the MHC is only one part of the immunogenetic complex of vertebrates. The failure to consider the complexity of the immune system limits our understanding of how selective pressures shape variation in resistance to pathogens (Acevedo-Whitehouse & Cunningham 2006). In certain instances, non-MHC genes are far more important to pathogen resistance than MHC genes (e.g. quantitative trait loci for helminth resistance have been identified on multiple chromosomes in small rodents; Behnke et al. 2003). So while variation at MHC genes is regarded as a good measure of the potential level of pathogen resistance in a host, one must be careful to not equate it with a full understanding of immunocompetence (Acevedo-Whitehouse & Cunningham 2006).

Infectious Disease, Genetics, and Conservation

Infectious Disease and Wildlife Conservation

The spread of infectious diseases among wildlife populations is a growing concern in the conservation community due to the potential for pathogen-mediated extinction events (Smith et al. 2009). While pathogen records for wild populations exist for less than half of all carnivores,

primates, and artiodactyls categorized as *critically endangered* or *endangered* by the IUCN, developments in disease modeling and epidemiological theory are allowing biologists to examine the potential implications of infectious disease on wildlife populations (Andersen & May 1992; Pedersen et al. 2007; Smith et al. 2009;).

Situations where pathogens are density-dependent are not likely to cause extinctions. This is because pathogens will face local 'fade-outs' as populations decrease (McCallum et al. 2001; Smith et al. 2009). Frequency-dependent pathogens, on the other hand, have the potential to drive a species towards extinction (de Castro & Bolker 2005). Pathogens transmitted as a function of frequency of infected individuals are not limited by population density thresholds, and can therefore still operate in extremely small populations (Smith et al. 2009). In some cases, a pathogen can mimic a frequency-dependent model if the population is structured spatially by social behavior or territoriality (O'Keefe & Antonovics 2002). Reservoir hosts can also pose a serious risk to wildlife populations; even if density-dependent pathogens fade out of a population, a nearby reservoir can re-introduce the pathogen freeing it from 'one host-one pathogen' infection dynamics (Smith et al. 2009). Perhaps most importantly, populations which are small and fragmented can experience heightened extinction risk due to the increased likelihood of stochastic events, which together with a concomitant decrease in genetic variability would heighten susceptibility to infectious diseases (Lyles & Dobson 1993; McCallum & Dobson 1995).

While the spread of existing pathogens is of high concern, emerging infectious diseases (EIDs) also pose a great threat to biodiversity, as well as human health (Daszak et al. 2000). The number

of wildlife EIDs has increased over the last few decades, due in part to the host-pathogen continuum between humans, domesticated animals, and wildlife (Figure 1.2) (Daszak et al. 2000). The emergence of a disease is generally linked to a change in the ecology of either host or pathogen (Schrag & Wiener 1995). Humans have greatly altered their ecology through population growth, encroachment into natural habitat, and international movement of domesticated species (Daszak et al. 2000). A number of anthropogenic drivers which lead to emergence of infectious diseases and the spread of existing pathogens have serious implications for wildlife populations (Daszak et al. 2000; Smith et al. 2009). Climate change, environmental pollutants, habitat alteration, and overexploitation are just some of the human-induced drivers which may heighten the probability of disease-induced wildlife population declines (Smith et al. 2009).

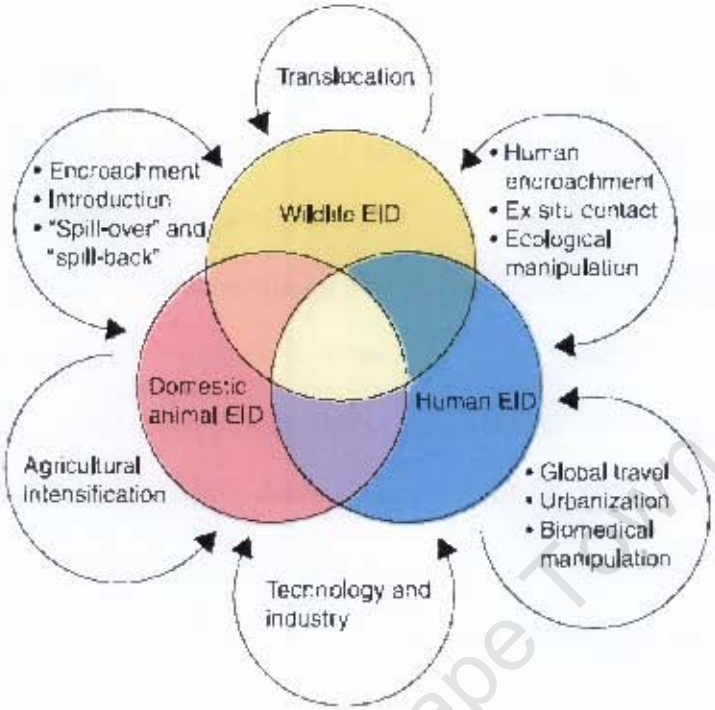


Figure 1.2: Visual representation of the host-pathogen continuum between humans, wildlife, and domesticated animals in which most EIDs are born. (Taken from Daszak et al. 2000).

Climate is predicted to change over the next century, with shifts in precipitation, climatic variability, and further warming of the globe (IPCC 2007). Shifts in climate are likely to alter pathogen distributions, transmission, survival rates, and host susceptibility (Harvell et al. 2002). For example, chytridiomycosis, a fungal infection caused by *Batrachochytrium dendrobatidis*, has caused significant declines of amphibian populations globally (Berger et al. 1998; Morrell 1999) and it is suggested that the global epidemic is linked to changes in climate, more specifically local increases in rainfall and temperature (Pounds et al. 2006; Bosch et al. 2007; Kriger et al. 2007).

The spread of invasive species is also having a large impact on the spread of pathogens globally. Part of the reason alien species do so well in new environments is because they are generally released from more than 75% of the pathogens found in their natural range (Torchin & Mitchell 2004). The pathogens that succeed in becoming established along with the invasive species pose a serious threat to the native wildlife, as they are usually novel pathogens in that environment (Smith et al. 2009). Non-native species can introduce pathogens through a variety of channels including food crops, timber, landfill waste, ballast water, and the global wildlife trade (Daszak et al. 2000; Smith et al. 2009). In 2007, it was determined there were over 300 species of non-native, live animals regularly imported to the United States alone which were considered to pose an ecological or economic threat (Jenkins et al.).

Environmental pollutants also pose a risk to wildlife and humans not only because of their general toxicity, but because of their role in pathogen spread. Certain pollutants are now thought to alter immunocompetence, leaving organisms more susceptible to disease (Smith et al. 2009). Laboratory animals have been found to suffer from a decrease in the function of humoral and cellular immunity due to exposure to organochlorines (Ahmed 2000), and wild marine mammals experience immunotoxicity and disease outbreaks after exposure to polychlorinated biphenels and other organic pollutants (Hammond et al. 2005).

Habitat alteration is considered the most important factor behind global biodiversity loss, and coupled with disease it has an enhanced capacity to influence population decline (Wilson 1992). Once habitat is lost, species are generally restricted in terms of movement and dispersal. This means contact among individuals increases, as does the spread of pathogens (Scott 1988).

Habitat alteration also fragments the landscape, opening more areas up to human exploitation. The bushmeat trade, for example, has resulted in the exchange of disease between human and non-human primates in Africa reflecting the ease with which pathogens can jump species. Diseases spread easily when species in contact have recent shared phylogenetic ancestry (Davies & Pedersen 2008; Smith et al. 2009). Indeed, humans share nearly 75% of their zoonotic EIDs with wild primate reservoirs (Woolhouse & Gowtage-Sequeria 2005).

Pathogen transfer occurs in both directions though, and there are multiple cases where human contact with non-human primates is leading to population decline. Mountain Gorillas (*Gorilla beringei)* have contracted measles from tourists (Daszak et al. 2000), common Chimpanzees (*Pan troglodytes*) of the Gombe National Forest in Tanzania had poliovirus introduced to their population (Goodall 1988), and the overall central African populations of both species have been reduced by 50% due to Ebola transmission (Leroy et al. 2004). The Chimpanzee population of the Tai National Park in Cote d'Ivoire was also decreased by nearly half due to two Ebola outbreaks and Anthrax infections (Formenty et al. 1999; Smith et al. 2009).

Domestic animal stocks also threaten the health of wildlife, as nearly 80% of their pathogens can infect wild populations (Cleaveland et al. 2001). Spillover of canine distemper virus from domestic populations of dogs continues to devastate wild populations of African wild dog (Carpenter et al. 1998), Black-footed ferret (Williams et al. 1988), and Spotted hyena in Tanzania (Haas et al. 1996). Movement of domesticated species internationally has also led to crippling EIDs such as rinderpest in Africa and bovine spongiform encephalitis (Daszak et al. 2000).

Adaptive Genetic Variation and Wildlife Conservation

Risk of pathogen-mediated population decline is not only influenced by emerging diseases and anthropogenic drivers, but also by the genetic make-up of hosts (Smith et al. 2009). Inbred populations are particularly at risk since the proportion of homozygous loci is likely to increase, increasing the frequency at which recessive mutations are expressed with each subsequent generation (Smith et al. 2009). In addition, studies have shown that inbreeding is correlated with decreased immunocompetence, greater susceptibility to disease, higher pathogen loads, and greater disease severity in wildlife populations (e.g. *Ovis aries*, Coltman et al. 1999; *Geospiza fortis and G. scandens*, Markert et al. 2004; *Drosophila melanogaster,* Spielman et al. 2004). Free-ranging populations that face geographic restrictions to dispersal due to habitat fragmentation may suffer the most in the long-run. Diverse genomic regions tasked with pathogen recognition, such as the Major Histocompatibility Complex, are constantly adapting to suites of rapidly evolving pathogens (Sommer 2005; Acevedo-Whitehouse & Cunningham 2006) such that geographic isolation could result in immunologically naïve populations who are more likely to experience rapid decline if novel pathogens are introduced (Smith et al. 2009). Interventional management of wildlife at risk of pathogen-mediated declines can be accomplished through a variety of channels including immunization, the creation of buffer zones between wild and domestic populations, as well as the development of translocation programs centered on enhancing levels of within-population genetic diversity (Smith et al. 2009).

The scope of human impact (e.g. habitat loss and fragmentation, urbanization, pollution) is unprecedentedly large, and has a range of impacts on the genetics and ecology of both vertebrate

and parasite populations (Sommer 2005). Human-induced changes in the environment can lead to shifts in the distribution and transmission of parasites and pathogens (Alitzer et al. 2003; Mitchell et al. 2005; Woolhouse et al. 2005) and as the human population is likely to continue expanding and exploiting, spread and emergence of infectious diseases with harrowing consequences for wildlife populations becomes increasingly likely (Sommer 2005). As variation at MHC loci is central in the development of pathogen resistance, and thus individual fitness and the long-term persistence of a species, conservation biologists can utilize studies of this adaptive immunity gene region to inform management of natural populations in uncertain and rapidly evolving pathogen environments (Hughes & Nei 1989; Takahata & Nei 1990; Sommer 2005).

Chapter 2

MHC-*DQA*1 Diversity in *Papio ursinus* of the Western Cape, South Africa

Introduction

Habitat fragmentation and loss via rapid transformation of land for urban development and agriculture are major forces driving global biodiversity loss (Wilson 1992; Rouget et al. 2003; McKinney 2002, 2006). Transformation of land negatively impacts the distribution and abundance of wildlife populations (Venier & Fahrig 1996; Gibbs 1998; Hargis et al. 1999; Best et al. 2001), species richness (Findlay & Houlahan 1997; Wettstein & Schmid 1999; Gurd et al. 2001; Steffan-Dewenter et al 2002), and genetic diversity (Gibbs 2001; Fahrig 2003). Habitat loss and fragmentation result in the loss of genetic diversity predominately through the interruption of dispersal behaviors (With & Crist 1995; Belisle et al. 2001; Tigas et al. 2002). Isolation of populations disrupts dispersal and thus genetic flow across the landscape, potentially intensifying the effects of genetic drift; as variation is lost from the population, inbreeding becomes more likely and may lead to decreases in reproductive success (Templeton et al. 1990; Frankham et al. 2002; Reed & Frankham 2003;). Inbreeding has also been strongly correlated with reduced immunocompetence, greater susceptibility to disease, higher pathogen loads, and greater disease severity in free-ranging populations (Coltman et al. 1999; Markert et al. 2004; Spielman et al. 2004). Due to these effects, fragmented populations with limited dispersal success may experience a heightened risk of population decline due to the increased likelihood of stochastic events and susceptibility to infectious diseases (Lyles & Dobson 1993; McCallum & Dobson 1995). Primate populations globally are suffering the consequences of habitat transformation (Orangutan, *Pongo pygmaeus*, Goossens et al. 2005; Cross River gorilla, *Gorilla*

gorilla diehli, Bergl et al. 2008), including the Chacma baboon (*Papio ursinus*) population of South Africa's Cape Peninsula.

Chacma baboons (*Papio ursinus*) range from the tip of South Africa all the way up into the Zambezi valley (Figure 2.1) and occupy a variety of landscapes including woodland, montane regions, subdesert, savannah, Fynbos, and Succulent Karoo (Groves 2001; Hoffman & Hilton-Taylor 2008). Troops are multi-female and usually have a multi-male hierarchy, except in smaller troops where there may only be one male, and average anywhere from 20 to 130 individuals in size (Hoffman & Hilton-Taylor 2008). Generally, female baboons are philopatric while males disperse to other troops (Alberts &Altmann 1995b).

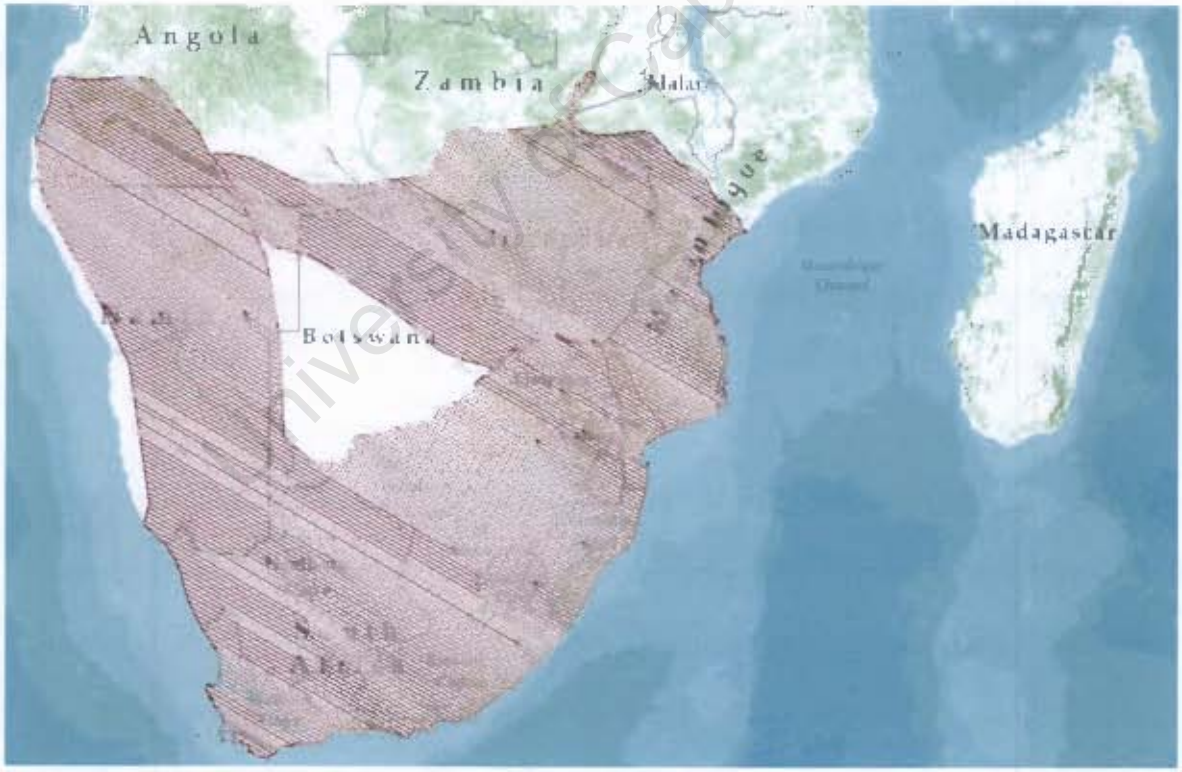


Figure 2.1: Current range of the Chacma Baboon (*Papio ursinus*) (map taken from IUCN Redlist. http://mapservices.iucnredlist.org/IUCN/mapper/index.html)

Throughout Africa baboon (*Papio*) populations overlap with humans and are generally perceived as pests (Hill 2000; Hoffman 2011). High levels of intelligence and dexterity result in highly effective crop-raiders and troops can thrive in human-modified environments (Strum 1994; Hill 2000). The population of Chacma baboons present on the Cape Peninsula, South Africa provides a prime example of the problems which can arise when non-human primates exist within human spaces. Baboons on the peninsula are well known for not only their typical crop-raiding behavior, but also for destruction of property and physical conflict with humans over food (Hoffman & O'Riain 2010; Hoffman 2011). Mediation of human-baboon conflict has been attempted through a number of methods such as electric fencing, sound aversion, and the employment of baboon monitors who physically drive baboons away from urban areas (Kansky & Gaynor 2000; van Doorn 2009; Hoffman 2011). Regardless of management attempts baboons and humans are continuously coming into contact on the peninsula, which sometimes has fatal consequences for the animals. Indeed, twenty-nine deaths caused by humans were recorded in 2008, resulting in a 7% loss of the peninsula's total population that year (Beamish 2010). In the face of constant conflict with humans, the baboon population has still managed to grow from 365 in 1998 to 460 in 2011 (Hoffman 2011).

Despite local population growth, there is still concern for the health of the Cape Peninsula's baboon population. Since the 15th century, nearly half of the Cape Peninsula's landscape has been altered and degraded by human activity, leading to a significant restriction in the historic geographic range of the baboon population (Hoffman 2011). Urbanization has created an impassable barrier for the baboons, completely isolating the peninsula's 12 extant troops from other populations in the Western Cape (Hoffman & O'Riain 2010). One outcome of this urban

Prior to any movement of animals, conservation biologists collect data outlined in the International Union for Conservation of Nature's (IUCN) guidelines for translocation to examine the appropriateness of translocation for their target population. The guidelines state that habitat and release site, species socioecology and behavior, financial and legal requirements, and genetic

obstruction has been the significant distortion of male reproductive behaviors. Prior to urbanization, male baboons would disperse from their natal groups to and from troops off the peninsula with a higher degree of success (Alberts & Altmann 1995a, 1995b). Gene flow was historically maintained across the landscape through dispersal of sub-adult males, so the inability of male baboons to properly disperse has had serious consequences for genetic variation within peninsula's baboons. Data from mitochondrial markers for baboons on the peninsula suggest that troop and population level genetic diversity is significantly lower than that of baboons living elsewhere in the Western Cape (Bishop et al., *unpub. data*). Haplotype diversity on the peninsula is minimal compared to other Western Cape baboons (on peninsula H*d* = 0.18; off peninsula H*d* = 0.56-0.92), with only four maternal lineages found among peninsula baboons where all troops are dominated by a single maternal haplotype (Bishop et al., *unpub. data*). These data suggest an uncertain future for the peninsula's baboon population, as inbreeding increases their chance of population decline due to stochastic events such as pathogen outbreaks (Smith et al. 2009). Risk of pathogen-mediated population decline is of particular concern given that organisms with shared phylogenetic ancestry are highly likely to exchange pathogens, and all 12 of the peninsula's baboon troops have some degree of contact with humans (Davies & Pedersen 2008; Ravasi 2009; Hoffman 2011). The magnitude of this risk is highlighted by recent findings whereby peninsula baboons possess antibodies reactive or cross-reactive to three viruses potentially of human origin: cytomegalovirus (CMV), hepatitis A virus (HAV), and Epstein-Barr virus (Drewe et al. 2012). Of twenty-seven baboons studied from five troops across the

genes make them an excellent surrogate for extensive disease screening as they provide evidence for whether populations have evolved in response to shared pathogen assemblages, and therefore whether or not translocation could be a feasible management option.

This study makes use of data from the MHC-*DQA* locus of Chacma baboons (*Papio ursinus*) in the Western Cape, South Africa to assess shared pathogen history among populations and inform a management approach that aims to ensure the persistence of an evolutionarily fit, and yet genetically isolated, population within the Cape Peninsula. In order to achieve this, the following questions were addressed: (1) Are levels of MHC-*DQA* gene diversity in Chacma baboons of the Western Cape, South Africa comparable to those reported in previous studies on free-ranging populations of non-human primates? (2) Is adaptive MHC gene diversity spatially clustered across the distribution of Chacma baboons in the Western Cape? and (3) Do phylogenetically related populations of Chacma baboons share immune diversity at the MHC-*DQA* locus that reflects their shared ancestry?

Methods

Sample Collection

The samples analyzed in this study were collected between 2006 and 2010 from individuals at 10 sites in the Western Cape, South Africa (Figure 2.2). A variety of sample types were collected for DNA analysis. Tissue biopsies from natural and traffic mortalities were collected into 96% ethanol and stored at 4° C until genomic DNA extraction. Fresh fecal samples were also collected during behavioral monitoring. These samples were placed in 96% ethanol and later stored in silica at 4°C until DNA extraction (Nsubuga et al. 2004).

Figure 2.2: Sites of Chacma baboon (*Papio ursinus*) tissue and fecal sampling in Western Cape, South Africa.

Genomic DNA Extraction and PCR Amplification

DNA was extracted from tissue samples using the DNeasy Blood and Tissue Kit (QIAGEN®) following the manufacturer's protocol. DNA extracted from fecal samples was isolated using the QIAamp DNA Stool Mini Kit (QIAGEN®) following manufacturer's protocol. All extractions were stored at -20°C. Primers targeting the second exon of the MHC Class II *DQA* locus (PCR

product size ~200 bp) were designed from Human (*Homo sapiens)* Leukocyte Antigen (HLA) sequences (HLA-GH26, 5'-GTGCTGCAGGTGTAA ACTTGTACCAG-3' and HLA-GH27, 5'-CACGGATCCGGTAGCAGC GGTAGAGTT G-3') (Scharf et al. 1986; Alberts 1999). Optimization of PCR conditions for genomic DNA was performed in a final reaction volumes of 30 µl containing 0.5pmol/µl forward and reverse primers, 0.2mM dNTPs, 1.5mM $MgCl_2$, 1 unit KAPA© taq (Applied Biosystems), 1x KAPA© taq buffer with dye (Applied Biosystems), and 20-50 nanograms of genomic DNA. PCR conditions were optimized by altering annealing temperature, number of PCR cycles, $MgCl_2$ concentration, and amount of genomic DNA. PCR was performed on ABI GenAmp 2700 thermocycler (Applied Biosystems) with the following standard cycling conditions: 94°C for 5 minutes; 30 cycles of 94°C for 30 seconds, 51°C for 30 seconds, 72°C for 30 seconds, 72°C for 10 minutes. PCR products were then electrophoresed on 2% agarose gels (SeaKem® LE). The correct size product was excised from for gel PCR purification.

MHC Cloning and Sequencing

Samples were cloned and sequences at the Central Analytical Facility, University of Stellenbosch. Purified PCR products were cloned using the pGEM®-T Easy Vector (Promega©) following the manufacturer's protocol and transformed into JM109 High Efficiency Competent Cells (Promega©). Positive clones were then sequenced in both directions using the BigDye v3.1 cycle sequencing kit (Applied Biosystems) and analyzed on the ABI 3130 Genetic Analyzer (Applied Biosystems).

Data Analysis

Sequence Alignment and Genetic Diversity

The resulting sequences were edited using BioEdit Sequence Alignment Editor v7.0.9.0 (Hall 1999) and aligned using ClustalW v1.4 (Thompson et al. 1994). Sequences with ambiguous nucleotides were removed from the data set. Gene identity (MHC-*DQA*) of the sequences was confirmed by GenBank BLAST searches (http://www.ncbi.nlm.nih.gov/genbank) and unique alleles were classified using DnaSP Sequence Polymorphism software v5.10.01 (Rozas et al. 2009). Sequences were then translated in BioEdit v7.0.9.0 (Hall 1999) and those with stop codons or multiple deletions were removed from the data set. Sequences in this study were considered unique if they were identified from PCR reactions from more than one individual or multiple PCR clones in one individual. For sequences found only once in one individual, they were only considered unique if they displayed more than one amino acid change or an amino acid change at a codon site which was altered in multiple sequences. Overall and per site nucleotide diversity was estimated in DnaSP v5.10.01 (Rozas et al. 2009). The number of unique alleles and their relative frequency was determined at each site and represented using pie graphs.

Phylogenetic Relationships

Phylogenetic relationships among Chacma baboon (*Papio ursinus*) MHC-*DQA* alleles and MHC-*DQA* alleles reported in other primates were determined using neighbor-joining method (Saitou & Nei 1987) in MEGA v5.05 (Tamura et al. 2011) using Nei-Gojobori genetic distance (Nei & Gojobori 1986) with Jukes-Cantor correction (Jukes & Cantor 1969). The analysis included the unique sequences identified in this study together with sequences from *Gorilla gorilla* (AF031277), *Pongo pygmaeus* (M76220), *Homo sapiens* (U85035), *Pan troglodytes*

(M76189), *Hylobates lar* (M76210), *Papio cynocephalus* (AF110841, AF131888, D88625), *Papio papio* (AF110842, AF110843, AF110844), *Papio hamadryas* (M76224, M76226, D89529), *Papio anubis* (AF110837, D88643), *Theropithecus gelada* (D88550), *Lophocebus aterrimus* (D88579), *Colobus guereza* (D88578), *Cercopithecus mitis* (D88679), *Cercopithecus aethiops* (M76187), *Cercopithecus neglectus* (D88580), *Macaca arctoides* (M76205), *Macaca fascicularis* (M76208), *Macaca mulatta* (M76202), *Sanguinus oedipus* (AY013374), and *Callithrix jacchus* (AF004741).

Patterns of Selection

To test whether the MHC-*DQA* locus of Chacma baboons has evolved under positive selection, the relative rate of synonymous (d_s) to non-synonymous (d_n) substitutions per site was calculated codon using the Selecton Server v2.4 (http://selecton.tau.ac.il/) (Stern et al. 2007; Doron-Faigenboim et al. 2005). The strength and type of selection was also examined at each individual codon using the Selecton Server v2.4 (http://selecton.tau.ac.il/) (Stern et al. 2007; Doron-Faigenboim et al. 2005). The Selecton Server v2.4 implements the model based approach of Yang et al. (2000) within a Bayesian framework. By assuming a prior distribution to account for heterogeneous d_n/d_s values among sites, the program tests for positive selection operating on the proteins against an assembly of evolutionary codon models including positive, neutral, and purifying selection. Because of the high rate of false positive when testing for positive selection at amino acid codons (Shriner et al. 2003), the likelihood under a model of positive selection was then compared to a model which doesn't allow positive selection using a likelihood ratio test implemented in Selecton v2.4.

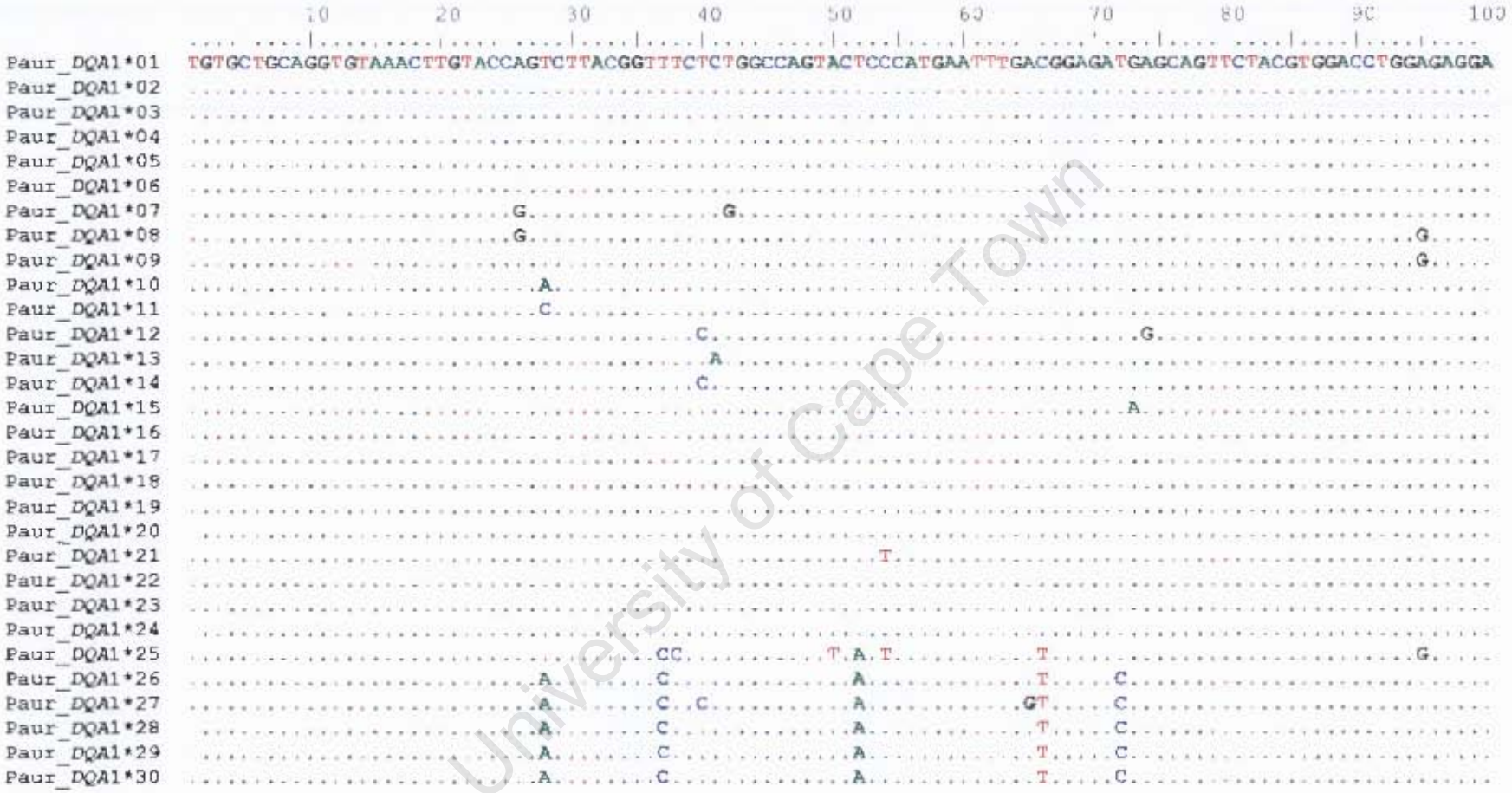
Results

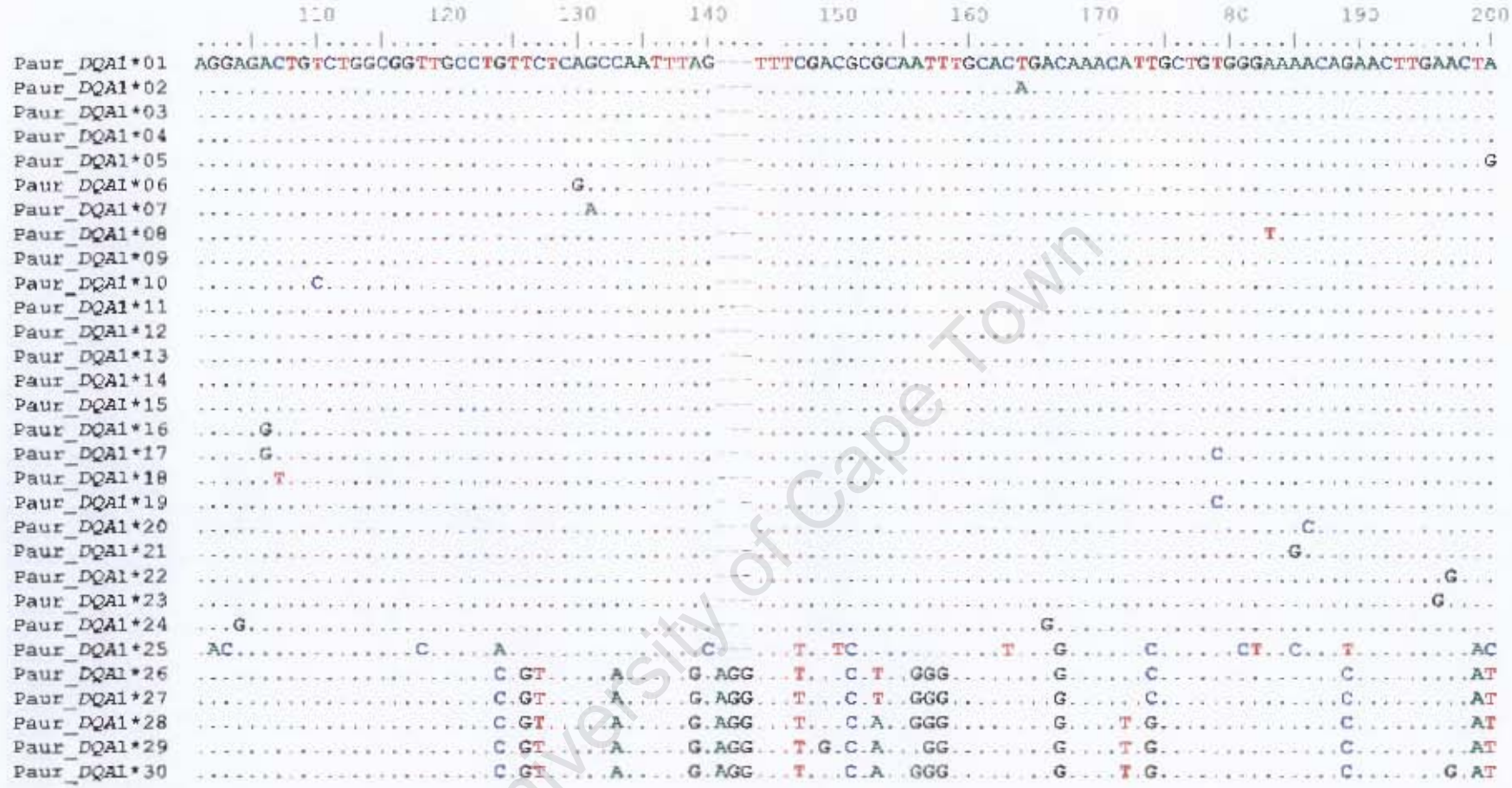
Characterization of MHC-DQA1 Sequences

From 41 individuals, 30 functionally unique Chacma baboon (*Papio ursinus*) MHC-*DQA* locus 1 sequences were identified (Figure 2.3). An NCBI nucleotide BLAST search confirmed homology of the sequences to MHC Class II α chain genes and showed high sequence similarity with exon sequences from crab-eating macaques (*Macaca fascicularis*), rhesus macaques (*Macaca mulatta*), yellow baboons (*Papio cynocephalus*), hamadryas baboons (*Papio hamadryas*), blue monkeys (*Cercopithecus mitis*), humans (*Homo sapiens*), and many other primate species. Five of the 30 unique sequences have a three base pair insertion (AGG, sites 141-143) (Figure 2.3) which results in a functional insertion of two amino acids (GG) (Figure 2.4). Following Kenter et al. (1992) the 30 sequences appear to belong to the first locus of the primate *DQ* α chain, and represent a number of different allelic lineages. Distinction between allelic lineages is made based on several amino acid motifs and changes reported in previous work on the evolutionary relationships between primate MHC-*DQA*1 and *DQA*2 alleles (Kenter et al. 1992). In the 30 *Papio ursinus* sequences described in this study, the presence of two separate allelic lineages from a single locus is confirmed by amino acid motifs from positions 41 -54 and the codon deletions at positions 52-53 (Figure 2.4) (Kenter et al. 1992). In addition to sequence motifs, the lineages can be separated by changes in specific amino acid residues: at site 57, Phenylalanine to Glycine and at site 61, Threonine to Arginine (Figure 2.4) (Kenter et al. 1992). From this information, we can determine that sequences Paur_*DQA*1*01-Paur_*DQA*1*25 likely belong to the first allelic lineage (MHC-*DQA*1-I), while Paur_*DQA*1*26-Paur_*DQA*1*30 belong to the second allelic lineage (MHC-*DQA*1-II, MHC-*DQA*1-III, MHC-*DQA*1-IV) (Figure 2.4) (Kenter et al. 1992).

The 30 sequences isolated in this study were designated Paur_*DQA*1*01-30 following the proposed nomenclature for MHC in nonhuman species; Paur is the species designation at MHC loci in *Papio ursinus*, *D* stands for the Class II locus, *Q* for the particular family of Class II genes, and *A* for genes that encode the receptor's α chain (Ellis et al. 2006). The overall level of nucleotide diversity was relatively high ($\pi = 0.045$), but the level was variable across the spatial distribution of sampling sites (Table 2.2; range $\pi = 0.006 - 0.100$). High levels of both nucleotide and amino acid sequence polymorphism were detected. A total of 65 of 243 (26.7%) nucleotides and 35 of 81 (43.2%) amino acid residues were polymorphic. At each variable site, a maximum of four different amino acid codons were observed (Figure 2.4). Eight of the twenty antigen binding site codons were polymorphic and 27 of the non-ABS codons were polymorphic, suggesting there is positive selection across the gene (Figure 2.4) (Brown et al. 1993).

Figure 2.3: Nucleotide sequence alignment for MHC-*DQA1* alleles of *Papio ursinus* populations in the Western Cape, South Africa. (N = 41 individuals)





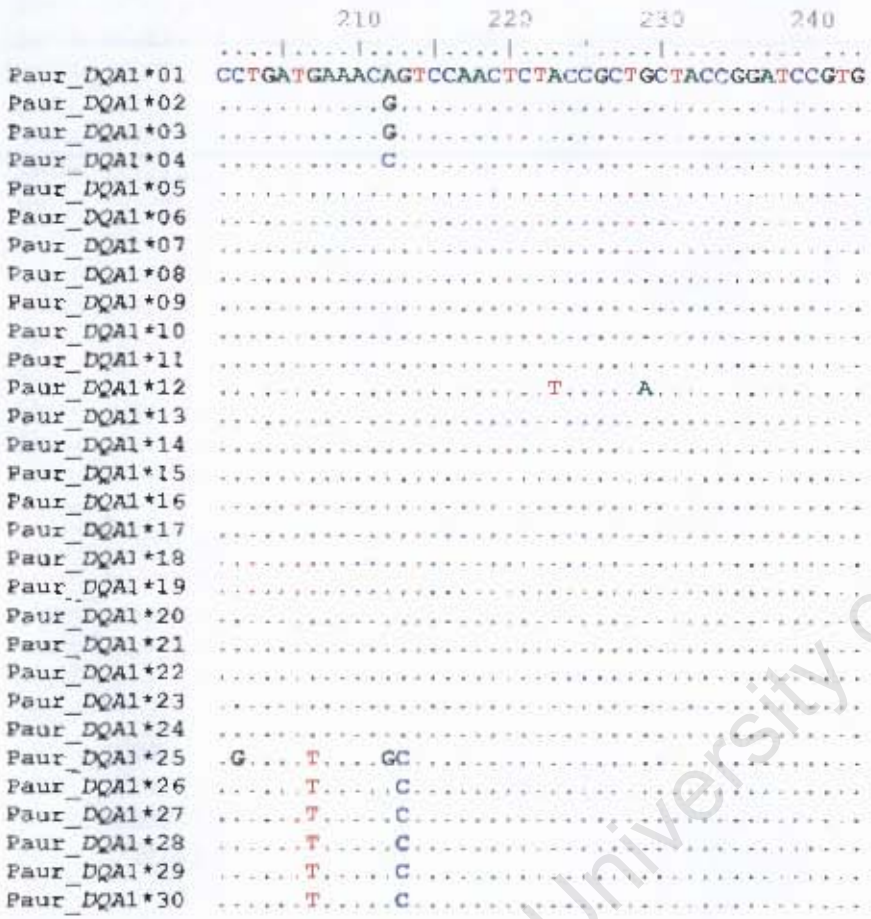
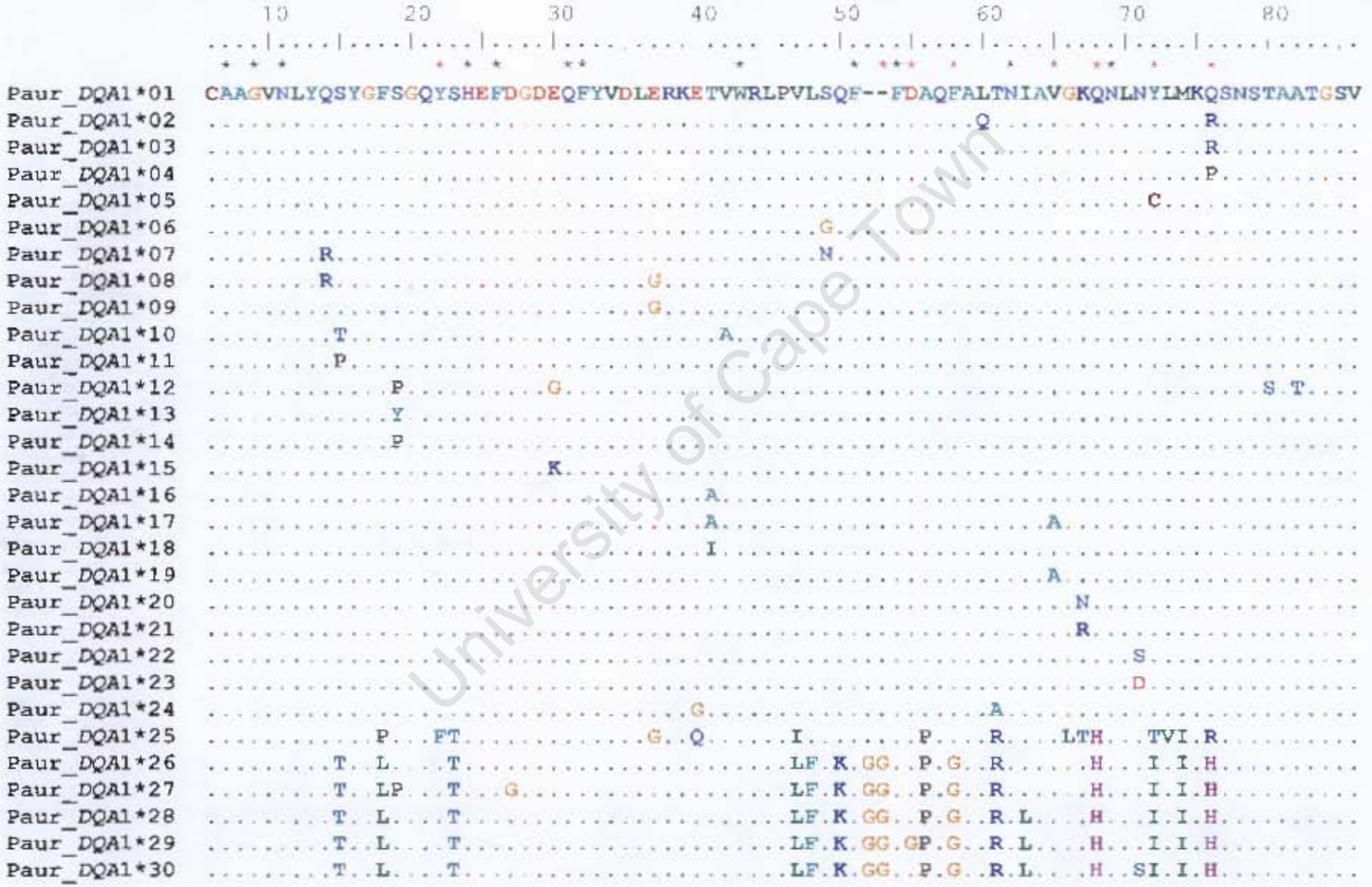


Figure 2.4: Amino acid sequence alignment for MHC-*DQA1* alleles identified in *Papio ursinus* populations of the Western Cape, South Africa. Numbers indicate the amino acid position in the MHC α chain; * indicates codons within the antigen binding site (ABS) (after Brown et al. 1993); red * indicate codons of the ABS with polymorphisms present. (N = 41 individuals).



In order to see if the number of unique alleles recovered from this study was typical, allele diversity was compared to other studies of primate the MHC-*DQA* locus 1. Note, the sample size (N=41) of this study was considerably larger than other studies of the primate MHC-*DQA*1 locus (Table 2.1). The retrieval rate of unique alleles per individual was comparable to that of most other studies (>50%). The only other study of MHC-*DQA*1 of free-ranging *Papio* populations returned a higher rate of unique alleles per individual (100%) than this study (73%) (Table 2.1) (Alberts 1999); however, their sample size was considerably smaller.

Species	Location	Number of individuals	Number of *DQA*1 alleles	Accession numbers
Pan troglodytes	Laboratory	6	4	(M76189-M76191, M76193)
Macaca mulatta	Laboratory	16	12	(M76194-96, M76199, M76200-03, M76209, M76228-30)
Cercopithicus mitis	Laboratory	17	7	(D88675 – D88681, D88684)
Papio papio	Captive (Zoo)	15	4	(AF110842 – AF110845)
Papio cynocephalus & Papio anubis	Free-ranging (Amboseli National Park, Kenya)	9	9	(AF110834-41, AF131888)
Papio ursinus	Free-ranging (Western Cape, South Africa)	41	30	(Not Yet Cataloged)

Table 2.1: Comparison of *Papio ursinus* MHC-*DQA*1 allelic diversity with other primates. (*Pan troglodytes, Macaca mulatta,* Kenter et al. 1992; *Cercopithecus mitis,* Mwenda et al. 1997;*Papio papio, Papio cynocephalus, Papio anubis,* Alberts 1999)

Phylogenetic Relationships

Phylogenetic relationships at the MHC-*DQA*1 locus among a number of primate species was investigated using the neighbor-joining (NJ) algorithm. The placement of Chacma baboon MHC-*DQA*1 alleles relative to previously reported data from primates is represented in Figure 2.5. The tree confirms sequence idenity of primate MHC-*DQA*1 and suggests the presence of more than one allelic lineage in the MHC-*DQA*1 locus of Chacma baboons (Figure 2.5). Allelic sequences cluster into the two separate lineages based on shared amino acid motifs (Figure 2.5) (Kenter et al. 1992) and these are seen very clearly in the unrooted NJ tree in Figure 2.6, showing sequences only from *Papio ursinus*.

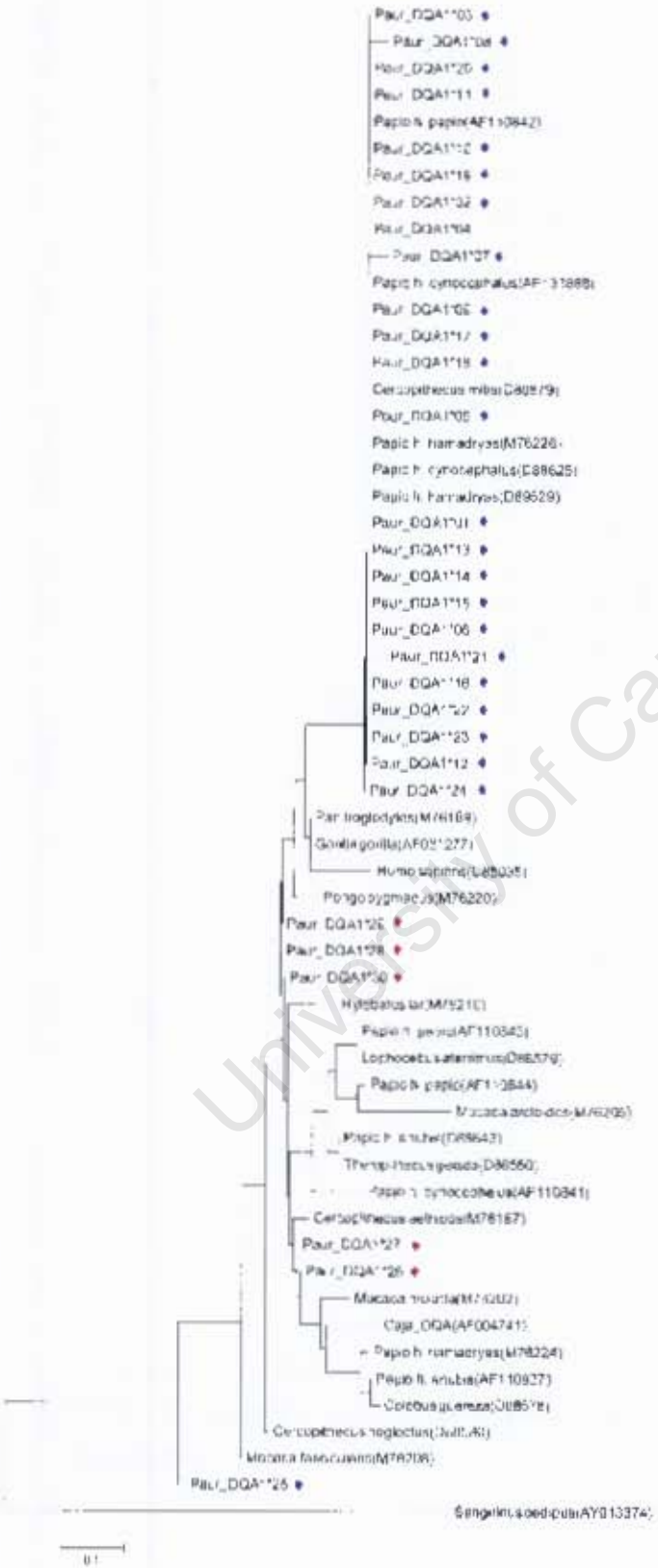


Figure 2.5: Phylogenetic relationships between *Papio ursinus* MHC-*DQA*1 alleles isolated in this study and a variety of other primate species. The scale bar at the bottom represents genetic distance. Blue diamonds identify sequences belonging to the first *DQA*1 allelic lineage of primates and pink diamonds identify sequences belonging to the second allelic lineage as per Kenter *et al* 1992).

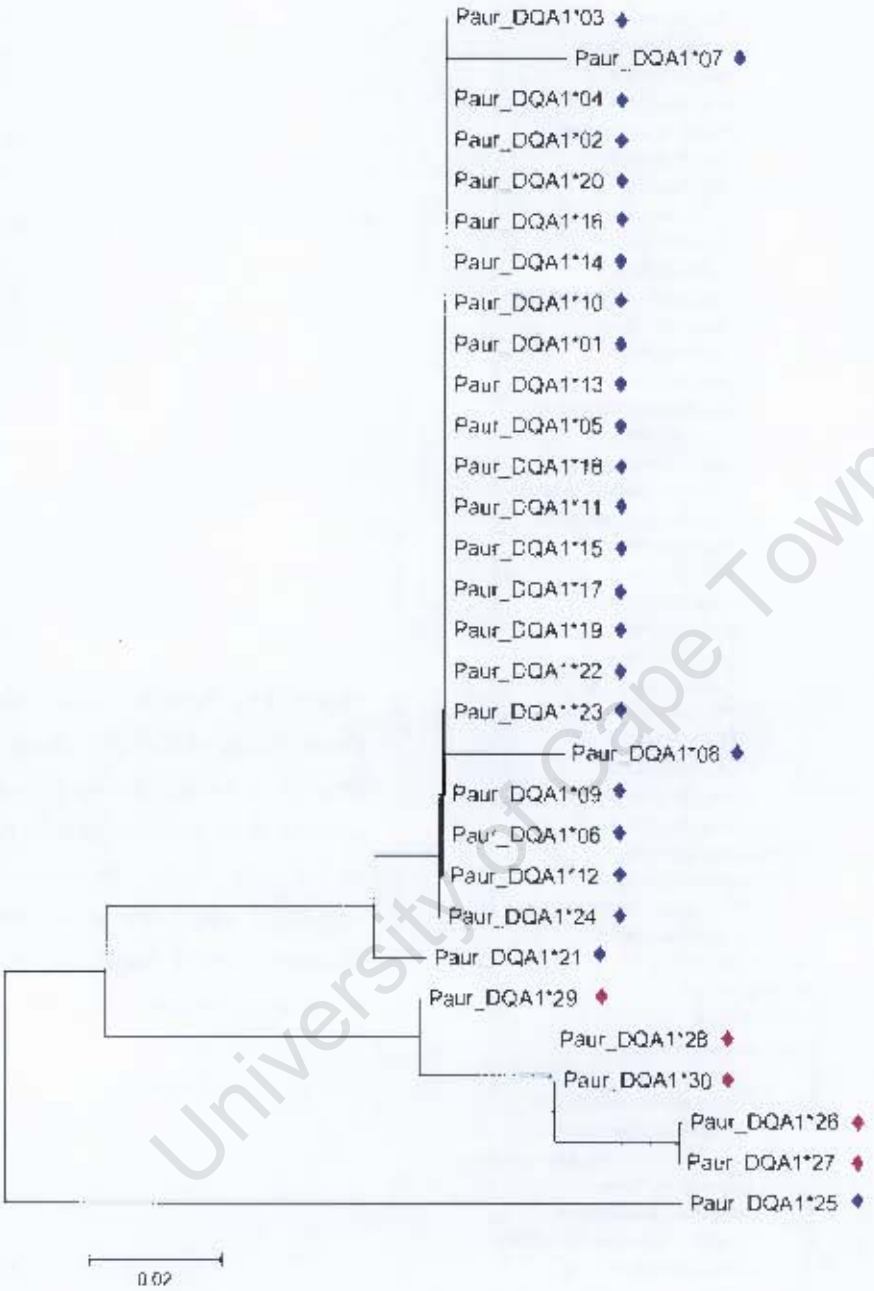


Figure 2.6: An unrooted tree showing the phylogenetic relationships between the 30 *Papio ursinus* MHC-*DQA1* alleles identified in this study. The scale bar represents genetic distance. Blue diamonds identify sequences belonging to the first *DQA1* allelic lineage of primates and pink diamonds identify sequences belonging to the second allelic lineage as per Kenter *et al* (1992).

Patterns of Selection

The substitution rates d_n and d_s were calculated to test whether variation at the MHC-*DQA1* locus of Chacma Baboons is maintained by positive selection. Figure 2.7 shows the d_n/d_s ratio per codon, and indicates the varying patterns of selection operating at each codon across the exon. D_n/d_s ratios above 1 are considered positive selection, values of 1 neutral, and anything below one represents purifying selection. The graph demonstrates that there is a mixture of balancing and purifying selection, suggesting positive selection plays a role in maintaining polymorphism at the MHC-*DQA1* locus of Chacma baboons. Figure 2.8 summarizes the relative levels of positive selection, neutral evolution, and purifying selection operating at each codon, and highlights that 8 of the 20 antigen binding sites are under the influence of strong positive selection. When the M8 model allowing for positive selection is tested against a null model (M8a) which does not allow for positive selection, the difference between the two models demonstrated that the level of positive selection acting on the MHC-*DQA1* locus in Chacma baboons is highly significant (M8 = -782.295; M8a = -788.005; p=0.001).

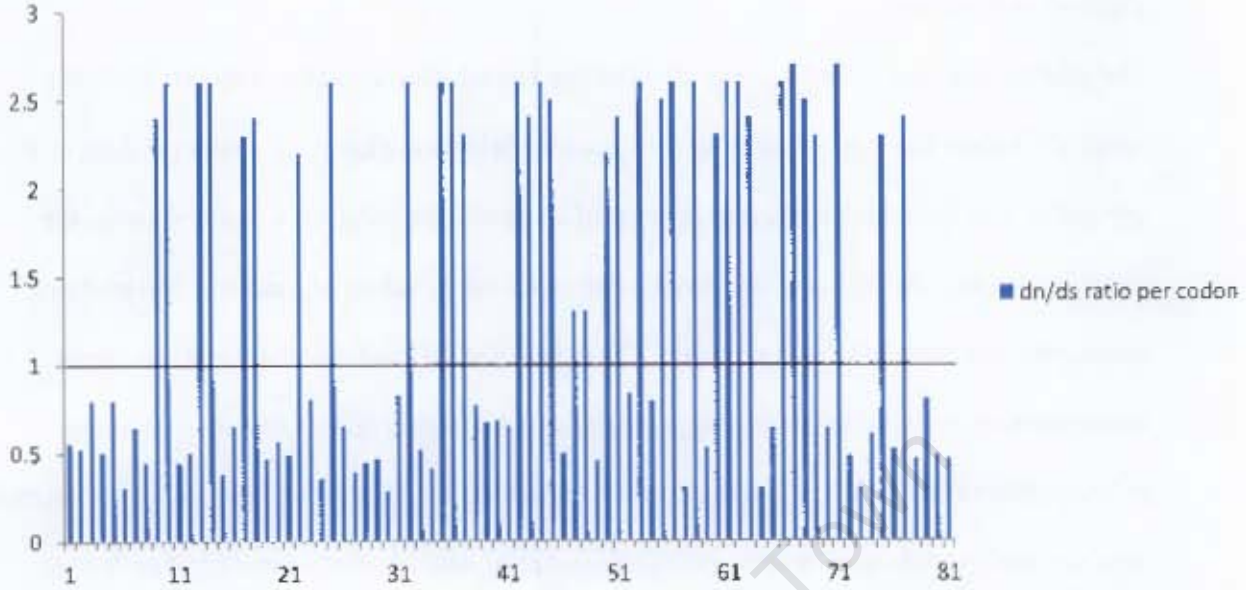


Figure 2.7: Rates of d_n and d_s substitution per codon for *Papio ursinus* MHC-*DQA*1 alleles. Values >1 represent positive selection, values = 1 represent neutral selection, and values<1 represent purifying selection.

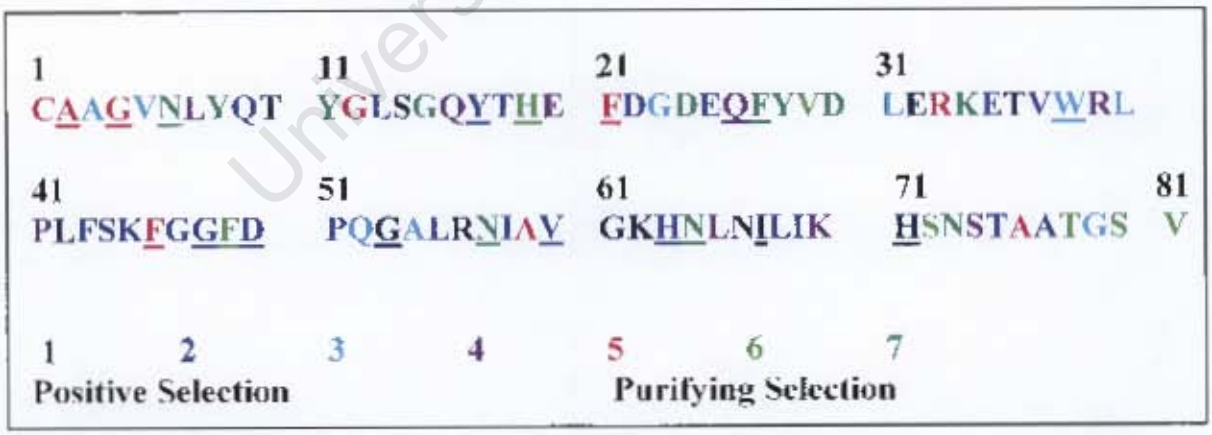


Figure 2.8: Type and strength of selection acting on each codon of *Papio ursinus* MHC-*DQA*1 alleles. Codons related to the antigen binding site (ABS) are underlined.

MHC Diversity across the Western Cape Landscape

Figure 2.9 visually represents the spatial composition of Chacma baboon MHC-*DQA*1 allelic diversity across a number of troops in the Western Cape of South Africa. Across the ten sampling sites, six of the 30 alleles occur at multiple sites (Paur_*DQA*1*01, Paur_*DQA*1*03, Paur_*DQA*1*05, Paur_*DQA*1*25, Paur_*DQA*1*26, Paur_*DQA*1*28) and only one allele occurs at all ten sites (Paur_*DQA*1*01). The dominant allele, Paur_*DQA*1*01, comprises anywhere from 43-92% of allelic diversity at any given site. Frequency of each unique allele per sampling site can be seen in Appendix 1.

Three alleles (Paur_*DQA*1*01, Paur_*DQA*1*26, Paur_*DQA*28) occur in populations both on and off the peninsula; and total diversity is almost equal with 15 unique alleles present among troops on the Cape Peninsula, and 18 unique alleles present among troops elsewhere in the Western Cape. The two distinct allelic lineages are represented in both Chacma baboons residing on the Peninsula and baboons residing outside the peninsula. While site by site nucleotide diversity is variable (Table 2.2; range $\pi = 0.006 - 0.100$), diversity across the landscape is roughly similar to overall nucleotide diversity of $\pi = 0.045$ (Table 2.2; Peninsula $\pi = 0.053$, Overberg $\pi = 0.037$, Heidelberg $\pi = 0.068$).

Location	Nucleotide Diversity (π)
Betty's Bay	0.100
Buffelsbay	0.006
Cape Point	0.009
Da Gama	0.064
Groot Olifantbos	0.081
Heidelberg	0.068
Pringle Bay	0.044
Rooiels	0.044
Slangkop	0.006
Tokai	0.091
All Peninsula	0.053
All Overberg	0.037
All Sites	**0.045**

Table 2.2: Nucleotide diversity (π) per each of the ten *Papio ursinus* MHC-*DQA1* sampling sites, as well as overall nucleotide diversity for the Cape Peninsula, Overberg, and all sites.

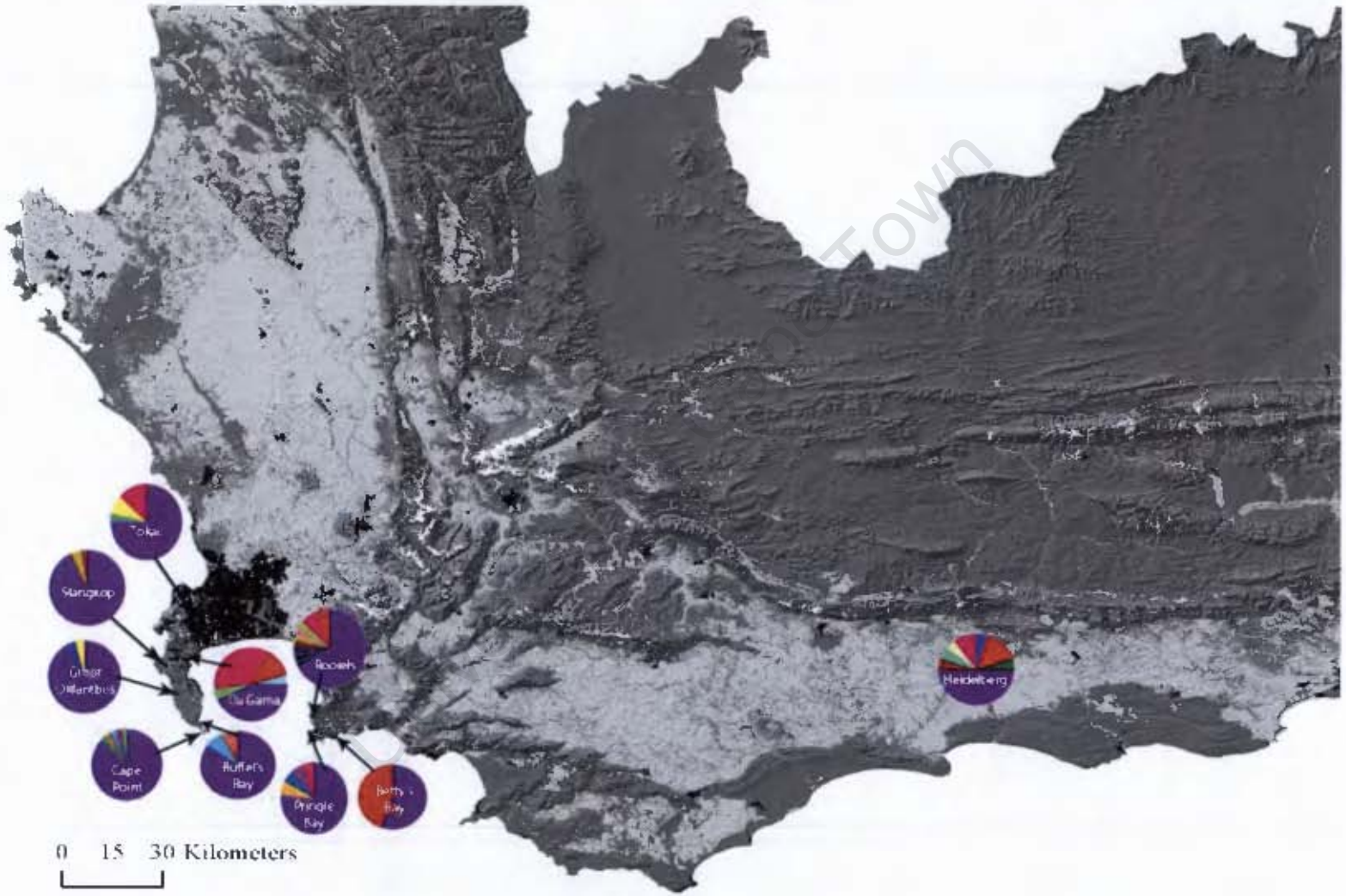


Figure 2.9: *Papio ursinus* MHC-*DQA1* diversity across the Western Cape, South Africa. (N = 41 individuals)

Discussion

As a result of extensive 20th century development and urban sprawl Cape Peninsula Chacma baboons have become completely isolated from populations elsewhere in the Western Cape, South Africa (Hoffman & O'Riain 2010); consequently, dispersing-age males on the Cape Peninsula can no longer successfully disperse from their natal groups to troops off of the peninsula, thus leading to the cessation of an important local and regional evolutionary process (Alberts & Altmann 1995a, 1995b). The geographic isolation of this population has thus without doubt influenced the low levels of genetic variation recently reported for Cape Peninsula troops relative to troops elsewhere in the Western Cape (Bishop et al., *unpub. data*). Loss of genetic variation due to inbreeding has been strongly linked to decreases in a multitude of reproductive fitness measures, as well as reduced immunocompetence, greater susceptibility to disease, higher pathogen loads, and greater disease severity in free-ranging populations (Coltman et al. 1999; Markert et al. 2004; Spielman et al. 2004). The consequences of limited dispersal for the Chacma baboons of the Cape Peninsula may therefore include a heightened risk of population decline owing to the increased likelihood of stochastic events and susceptibility to infectious diseases (McCallum & Dobson 1995; Lyles & Dobson 1993). Pathogen-mediated population decline is of special concern as baboons are highly likely to exchange pathogens with humans due to shared phylogenetic ancestry (Davies & Pedersen 2008) and there is already evidence of viral transmission, with several baboons on the peninsula expressing antibodies to viruses (cytomegalovirus, hepatitis A, Epstein-Barr) (Drewe et al. 2012) and parasites (*Trichuris trichiura*) (Ravasi 2009) of potential human origin.

The Cape Peninsula baboons are not only in the prime environment for pathogen spread, but also for the emergence of infectious disease. Habitat loss, environmental pollution, and the ever increasing close interface between humans, wildlife, and domesticated animals are all part of the current Cape Peninsula landscape; indeed these are all factors which have knowingly lead to the emergence of infectious diseases among wild animal and human populations in the past (Daszak et al. 2000; Smith et al. 2009). Active management of the Cape Peninsula's baboon population through translocation is proposed as one way to restore gene flow and ensure fitness, thus limiting the chances of pathogen spread and emergence (Moritz 1999; Fischer & Lindenmayer 2000). Whilst translocating individuals from troops off the peninsula into troops on the peninsula would undoubtedly introduce new genetic variation, and thereby provide the geographically isolated population on the peninsula with a greater chance of persistence, there are many risks involved. This is where an understanding of the adaptive immune response, in this case the major histocompatibility complex, is of crucial importance. The MHC-*DQA*1 locus diversity and spatial distribution data from this study allow us to investigate the similarity of the pathogen environments in which the isolated baboon populations have evolved (Sommer 2005). In so doing, this study provides insight into the shared spatial evolution of the alleles involved in the adaptive immune response of Chacma baboons and directly informs the discussion on the safety and feasibility of translocation as a management tool for this baboon population.

Chacma Baboon MHC: Diversity, Evolution, and Spatial Relationships

MHC-*DQA* gene diversity in Chacma baboons across the Western Cape, South Africa was found to be high with 30 unique functional alleles recovered from 41 individuals. The findings are comparable with those of other primate MHC-*DQA* studies carried out on both captive and free-

ranging populations. In a study of laboratory Olive baboons (*Papio anubis*), three unique alleles were recovered from five individuals (Mwenda et al. 1997); a free-ranging population of Yellow baboons (*Papio cynocephalus*) and Olive baboons (*Papio anubis*) which sits on the edge of a hybrid zone in Amboseli National Park, Kenya returned nine unique alleles from nine screened individuals (Alberts 1999). These *Papio* studies, along with data from other primate species, show that there is a general retrieval rate of 50% or greater for unique MHC-*DQA* alleles in primates. Free-ranging *Papio ursinus* populations of the Western Cape, South Africa appear to follow this trend.

The MHC-*DQA* gene of Chacma baboons in the Western Cape is consistent with Kenter *et al's* (1992) proposed model for the evolution of the primate *DQA* gene. Upon first inspection, the data revealed two very distinct groups of alleles (Figure 2.4); after phylogenetic analysis, it is clear that two separate allele lineages characterize the MHC-*DQA1* gene in *Papio ursinus*. Our findings reflect the results Kenter *et al* (1992) report in other Old World primate species, namely that one allelic lineage (coined MHC-*DQA*1-I) gave rise to a second group of alleles (MHC-*DQA*1-II, MHC-*DQA*1-III, MHC-*DQA*1-IV). This study also supports the suggestion that most Old World monkeys lack the MHC-*DQA*2 locus (*Macaca mulatta, Macaca arctoides, Macaca fascicularis, Papio hamadryas, Cercopithecus aethiops*, Kenter et al. 1992; *Cercopithecus mitis*, Mwenda et al. 1997), as no sequences resembling MHC-*DQA*2 were recovered from the extensive cloned library generated for *Papio ursinus* (Bontrop et al. 1989; Kenter et al. 1992).

Data concerning MHC gene diversity exists for a multitude of vertebrates and is usually examined in order to study adaptive gene evolution, and/or the impacts of habitat alteration and

population bottlenecks on adaptive genetic diversity (Kenter et al. 1992; Wenink et al. 1998; Aguilar et al. 2004). A large number of the studies on the evolution of primate MHC genes have utilized captive individuals and their main objectives have been to describe allelic diversity for a species, pair it with data from other species, and then examine the evolutionary histories of adaptive genes in primates (e.g. Kenter et al. 1992; Mwenda et al. 1997; Alberts 1999). To a lesser extent, studies of MHC diversity in free-ranging primate populations have been utilized to examine evolutionary relationships at MHC genes; one study on free-ranging Chacma baboons (*Papio ursinus*) of the Tsaobis Leopard Park, Namibia found evidence for trans-species inheritance of MHC-*DRB* genes in Old World primates (Huchard et al. 2006). However, most MHC studies utilizing DNA from free-ranging populations are concerned with examining the effects of habitat fragmentation and population bottlenecks on adaptive genetic diversity. It is well established that habitat fragmentation and bottlenecks may result in the loss of genetic diversity at neutral genes (Keller & Waller 2002; Reed & Frankham 2003), but many studies have shown that diversity at MHC genes can remain high in affected species (examples include African Buffalo, *Syncerus caffer caffer,* Wenink et al. 1998; San Nicolas Island fox, *Urocyon littoralis dickey*, Aguilar et al. 2004). Primates are no exception, and species such as Mouse lemurs (*Microcebus murinus*, Schad et al. 2004) and Gorillas (*Gorilla gorilla, Gorilla beringei*, Lukas et al. 2004) display high levels of MHC-*DRB* allelic diversity despite the negative influences of habitat loss and other anthropogenic drivers affecting population size and genetic diversity at neutral genes. These studies are crucial to understanding the process of maintaining diversity at MHC loci and suggest balancing selection and selection for functional diversity in the presence of pathogens are responsible for maintaining high levels of MHC polymorphism (Sommer 2005). Studies of MHC genes are also increasingly being used to identify specific

alleles involved in resistance to parasites and pathogens of interest in threatened primate species. For example, a study of Mouse lemur (*Microcebus murinus*) MHC-*DRB* diversity found that specific alleles are associated with levels of parasite load (high parasite load, *Mimu-DRB**1; low parasite load, *Mimu-DRB**6 and *Mimu-DRB**10) (Schad et al. 2005). Our study of *Papio ursinus* MHC-*DQA*1 follows an emerging trend in MHC research; the data is not only utilized to explore gene diversity, evolution, and patterns of selection, but also to inform conservation management of a species. Our findings are of particular interest as this appears to be the first large-scale and spatially explicit study of the MHC-*DQA*1 locus data in a free-ranging population of baboons, where despite relatively low levels of neutral mitochondrial diversity the isolated troops on the Cape Peninsula maintain comparable levels of MHC diversity to those outside the region.

The spatial distribution of adaptive MHC-*DQA*1 diversity, while suggesting some degree of geographical clustering, appears to be comparable across the distribution of Chacma baboon troops sampled in the Western Cape, South Africa. The only spatial clustering identified from this data set relates to three alleles (Paur_*DQA*1*03, Paur_*DQA*1*05, Paur_*DQA*1*25) which appear in more than one troop, but are restricted to troops on the peninsula; these alleles, however, occur at low frequencies suggesting they may not currently be as functionally important as other alleles (Sommer 2005). While allele diversity is variable across the landscape in terms of allele numbers (anywhere from 2 – 10 unique alleles per troop sampled), the allelic composition of this diversity is very similar across the landscape. A single allele dominates all troops (Paur_*DQA*1*01), comprising anywhere from 43-92% of the total diversity observed at any one site and both allelic lineages are represented among troops on and off the Cape

Peninsula. Functionally, this suggests that Chacma baboons across the Western Cape have a roughly equivalent adaptive immune allelic repertoire.

The shared functional diversity at the MHC-*DQA*1 locus of Chacma baboons across the Western Cape, South Africa indicates that phylogenetically related populations have evolved under the pressure of similar pathogen communities (Sommer 2005). In addition, the high levels of positive selection detected at the MHC-*DQA*1 locus of these baboons support the likelihood of pathogen-mediated evolution at the gene (Piertney & Oliver 2006). Having apparently evolved under the pressure of similar pathogen environments, the risk of introducing novel pathogens to the peninsula baboon population through the introduction of individuals from elsewhere in the Western Cape should be low (Sommer 2005; Piertney & Oliver 2006). As markers at neutral genes show significantly low levels of diversity (Bishop et al., *unpub. data*), the data from this study suggests that translocation bears strong consideration as a management tool for encouraging genome wide diversity in the Chacma baboon population of the Cape Peninsula. While preserving the baboons for the sake of them being the last of the historically occurring large mammals on the peninsula is enough incentive to manage the population for some conservation biologists, it is not the only legitimate reason the proposal of translocation requires serious consideration.

Economic and Health Incentives for Managing Chacma Baboons

Managing the Chacma baboon population of the Cape Town area for increased fitness not only helps maintain the well-being of the environment, but also the economy and health of the citizens. In 2007, the Cape Peninsula attracted 1.8 million tourists (City of Cape Town 2008),

making it the second largest tourist attraction in South Africa after the Kruger National Park (Macdonald & Cowling 1996; Hoffman 2011). Cape Point is a particularly popular tourist destination on the peninsula, and also home to some of Cape Town's most infamous baboons. The City of Cape Town recognizes that the Cape's baboon population is a large part of the tourist draw and therefore of significant economic value (Kansky & Gaynor 2000; City of Cape Town 2009). Economic value is not only tied to the charisma of the baboons, though.

As previously mentioned, several factors including the constant contact between humans and baboons create a prime environment on the Cape Peninsula for the emergence of infectious diseases and indeed humans share nearly 75% of their zoonotic EIDs with wild primate reservoirs (Woolhouse & Gowtage-Sequeria 2005). The emergence of infectious disease has obvious negative consequences for the health of both human and wildlife populations involved, but it has equally damaging economic costs. Treatment of Lyme disease in humans, a tick-born disease associated with White-tailed deer, costs the United States an estimated $500 million every year (Daszak et al. 2000) and when African horse sickness (AHS), a virus which continues to be a problem in South Africa, was imported into Spain, costs estimated at $20 million were required to contain the epidemic (Meltzer 1993; Mellor & Boorman 1995). The economic impacts of zoonotic EIDs are not always so easy to quantify and may have far reaching consequences. For example, people who resided in the UK for more than six months between 1980 and 1996 are not allowed to donate blood in the United States, as they are considered potential carriers of bovine spongiform encephalopathy (BSE). This is estimated to have decreased the U.S. blood supply by at least 2.2% (Ault 1999). In another case, the Nipah virus, which crossed into humans in 1999, had economic consequences for both the livestock market in

Malaysia and human health. Fruit bats are the reservoir species for Nipah virus, but when deforestation and drought in Malaysia resulted in a significant decrease in forest fruit production the bats moved to feed at pig farms. The result was an overwhelming loss of domesticated pigs, which also passed the disease along to livestock farmers and resulted in over 100 human casualties (Daszak et al. 2001; Chua et al. 2002).

The case of Nipah virus highlights another important aspect of zoonotic infectious disease emergence, namely the huge potential impact on human health. There is strong evidence that Human Immunodeficiency Virus (HIV), the virus which causes acquired immune deficiency syndrome (AIDS), passed into humans sometime during the twentieth century via a Simian Immunodeficiency Virus (SIV) mutant from non-human primates (Morens et al. 2004). AIDS is now predicted to have surpassed the death toll of the 1918-1920 influenza pandemic and the Black Death, both of which resulted in at least 50 million human deaths (Kohn 1995; Johnson & Mueller 2002). There are a host of other infectious diseases with ties to cross-transmission between humans and free-ranging primates, such as Ebola, Marburg virus, hepatitis and herpes B (Renquist & Whitney 1987; Daszak et al. 2000). While outbreaks of zoonotic diseases in human populations can be related back to economic impact, it is equally important to consider the ethical implications of EIDs.

Though the Cape Peninsula has many of the factors considered as primary initiators of pathogen spread and emergence, translocation as a management tool may help prevent not only population decline of the peninsula's Chacma baboons, but also any potential economic or health impacts related to baboon-mediated pathogen spread. The International Union for Conservation of Nature

(IUCN) recommends accumulating data on a number of biological factors (habitat and release site, socioecology, behavior, genetic and disease screening) before choosing translocation as a management tool (IUCN 1998; Goossens et al. 2002; Soorae & Baker 2002). There is already a wealth of behavioral and socioecological information on the peninsula's Chacma baboon population due in large part to the University of Cape Town's Baboon Research Unit, as well as extensive studies carried out on a successfully translocated group of Olive baboons (*Papio anubis*) (Soorae & Baker 2002; Strum 2005). As noted before, genetic screening has also already been carried out for Chacma baboon populations on and off the Cape Peninsula (Bishop et al., *unpub. data*); a thorough understanding of pathogen diversity and disease prevalence remains a major challenged (but see Drewe et al. 2012 for an initial study) and data from immunogenetic markers can provide complementary insight into the evolutionary and ecological importance.

In conclusion, data from the MHC-*DQA*1 gene obtained from this study reveal that populations of phylogenetically related Chacma baboons in the Western Cape, South Africa share a suite of functionally related MHC alleles, suggesting that evolutionary related populations have evolved in similar pathogen environments and therefore are likely to have corresponding adaptive immune response to disease. This study provides support for a management regime that includes assisted dispersal of animals from outside the peninsula into troops on the peninsula as an effective tool to ensure the persistence of adaptive immune genetic variability and genome wide diversity. In so doing, the movement of novel variation into the peninsula is highly likely to influence local population and troop-level fitness, and thus the long-term persistence of an evolutionarily fit population of Chacma baboons on the Cape Peninsula, South Africa

Chapter 3

Study Review and Synthesis

Complications with the Study

Some minor complications arose in the study due to utilizing non-invasive sampling techniques (fecal samples). We encountered slight difficulty owing to degradation of old dung samples which has also been reported for a number of MHC studies using non-invasive sampling (Knapp 2005b); for these degraded samples, the PCR reaction and conditions had to be repeatedly optimized. While the end products removed from the gel were high enough quality for successful cloning and sequencing, it was relatively costly in terms of time and we were unable to successfully amplify samples from the Cederberg region of the Western Cape. Additionally, the primers used in this study (HLA-GH26 and HLA-GA27) have been shown to amplify across a broad range of vertebrates at the MHC-*DQA* locus (Scharf et al. 1986; Alberts 1999) and a GenBank BLAST search of the primer sequences confirmed similarity to numerous genomes including bacteria; as a result some cloned products from fecal DNA were identified as bacterial in origin or from other organisms present in the dung. This problem was only observed in DNA sampled extracted from fecal material and did not happen often enough to negatively affect the quality of our dataset; these sequences were simply purged from the dataset.

Further Study

It would be interesting to have a spatially explicit model of the MHC-*DQA*1 locus across a greater extent of the South African landscape, as it would enable researchers to examine how the pathogen environment shifts across the country and perhaps have a better understanding of how the MHC-*DQA*1 locus has evolved in Chacma baboons. Samples from troops in the Cederberg

would be especially useful, as they represent another area that is phylogenetically the sister clade to baboon lineages on the Cape Peninsula and in the Overberg region, and thus could potentially be another source of individuals for translocation.

Data on the Class I MHC genes of baboons would also be a very valuable addition to the dataset. Since Class I genes are involved in the recognition and presentation of intracellular pathogens, they can provide greater insight into adaptive immunity against viruses in Chacma baboons. As the peninsula baboons have recently been found to carry antibodies against viruses of potential human origin (cytomegalovirus, hepatitis A, Epstein-Barr; Drewe et al. 2012), this could be extremely valuable data to inform management options.

References

Acevedo-Whitehouse, K., and A. Cunningham. 2006. Is MHC enough for understanding wildlife immunogenetics? Trends in Ecology and Evolution **21:**443-438.

Aguilar, A., G. Roemer, S. Debenham, M. Binns, D. Garcelon, and R. K. Wayne. 2004. High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. Proceedings of the National Academy of Sciences **101**(10)**:**3490-3494.

Ahmed, S. A. 2000. The immune system as a potential target for environmental estrogens (endocrine disrupters): A new emerging field. Toxicology **150:**191-206.

Alberts, S. C. 1999. Thirteen MHC-*DQA*1 alleles from two populations of baboons. Immunogenetics **49:**825-827.

Alberts, S. C., and J. Altmann. 1995a. Preparation and activation: Determinants of age at reproductive maturity in male baboons. Behavioral Ecology and Sociobiology **36**(6)**:**397-406.

Alberts, S. C., and J. Altmann. 1995b. Balancing costs and opportunities: Dispersal in male baboons. American Naturalist **145**(2)**:**279-306.

Alberts, S. C., and C. Ober. 1993. Genetic variability of the MHC: A review of non-pathogen-mediated selective mechanisms. American Journal of Physical Anthropology **36:**71-89.

Alitzer, S., D. Harvell, and E. Friedle. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology and Evolution **18:**589-596.

Altuvia, Y., and H. Margalit. 2004. A structure-based approach for prediction of MHC-binding peptides. Methods **24:**454-459.

Anderson, R. M., and R. M. May. 1992. Infectious diseases of humans, dynamics and control. Oxford University Press, New York.

Arkush, K. D., A. R. Giese, H. L. Mendonca, A. M. McBirde, G. D. Marty, and P. W. Hedrick. 2002. Resistance to three pathogens in the endangered winter-run Chinook salmon (*oncorhynchus tshawytscha*): Effects of inbreeding and major histocompatibility complex genotypes. Canadian Journal of Fisheries and Aquatic Sciences **59:**966-975.

Ault, A. 1999. USFDA advisory panel urges ban on UK blood. Lancet **353**(9169)**:**2050.

Averdam, A., C. Kuschal, and N. Otto. 2011. Sequence analysis of the Grey Mouse lemur (*Microcebus murinus*) MHC class II *DQ* and *DR* region. Immunogenetics **63**(2)**:**85-93.

Beamish, E. K. 2010. Causes and consequences of mortality and mutilation in baboons of the Cape Peninsula, South Africa. M.Sc. thesis. University of Cape Town, Cape Town, South Africa.

Beauchamp, G. K., and K. Yamakazi. 2003. Chemical signaling in mice. Biochemical Society Transactions **31:**147-151.

Behnke, J. M., F. Iraqi, D. Menge, R. L. Baker, J. Gibson, and D. Wakelin. 2003. Chasing the genes that control resistance to gastrointestinal nematodes. Journal of Helminthology **77:**99-110.

Behnke, J. M., and F. N. Wahid. 1991. Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): H-2 linked genes determine worm survival. Parasitology **103:**157-164.

Belisle, M., A. Desrochers, and M. J. Fortin. 2001. Influence of forest cover on the movements of forest birds: A homing experiment. Ecology **82:**1893-1904.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences **95:**9031-9036.

Bergl, R. A., B. J. Bradley, A. Nsubuga, and L. Vigilant. 2008. Effects of habitat fragmentation, population size and demographic history on genetic diversity: The Cross River gorilla in a comparative context. American Journal of Primatology **70:**848-859.

Bernatchez, L., and C. Landry. 2003. MHC studies in nonmodel vertebrates: What have we learned about natural selection in 15 years? Journal of Evolutionary Biology **16:**363-377.

Best, L. B., T. M. Bergin, and K. E. Freemark. 2001. Influence of landscape composition on bird use of rowcrop fields. Journal of Wildlife Management **65:**442-449.

Bontrop, R. E., N. Otting, L. A. M. Broos, M. C. Noort, M. Kenter, and M. Jonker. 1989. RFLP analysis of the *HLA-, ChLA-* and *RhLA-DQ* alpha chain gene regions: Conservation of restriction sites during evolution. Immunogenetics **30:**432-439.

Bosch, J., L. M. Carrascal, L. Duran, S. Walker, and M. C. Fisher. 2007. Climate change and outbreaks of amphibian Chytridiomycosis in a montane area of central Spain; is there a link? Proceedings of the Royal Society of London **274:**253-260.

Boyce, W. M., M. E. Weisenberger, M. C. T. Penedo, and C. K. Johnson. 2011. Wildlife translocation: The conservation implications of pathogen exposure and genetic hetereozygosity. BMC Ecology **11:**51-57.

Brown, J. H., T. S. Jardetzky, J. C. Gorga, L. J. Stern, R. G. Urban, J. L. Strominger, and D. C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-*DR*1. Nature **364:**33-39.

Brown, J. L., and A. Eklund. 1994. Kin recognition and the major histocompatibility complex: An integrative review. American Naturalist **143:**435-461.

Burke, R. L. 1991. Relocations, repatriations, and translocations of amphibians and reptiles: Taking a broader view. Herpetologica **47**(3):350-357.

Carpenter, M., M. Appel, M. Roelke-Parker, L. Munson, H. Hofer, M. East, and S. O'Brien. 1998. Genetic characterization of canine distemper virus in Serengeti carnivores. Veterinary Immunology and Immunopathology **65:**259-266.

Chen, C. Y., S. A. Cohen, M. B. Zaleski, and B. Albini. 1992. Genetic control of streptococcus-induced hepatic granulomatous lesions in mice. Immunogenetics **36:**28-32.

Chua, K., B. Chua, and C. Wang. 2002. Anthropogenic deforestation, El Nino and the emergence of Nipah virus in Malaysia. Malaysian Journal of Pathology **24:**15-21.

City of Cape Town. 2008. City Statistics. Available from http://www.capetown.gov.za/en/stats/Pages/City Statistic.aspx (accessed February 1 2012).

City of Cape Town. 2009. Baboon Management in Cape Town. Available from http://www.capetown.gov.za/en/MediaReleases/Pages/BaboonManagementinCapeTown.aspx (accessed February 5 2012).

Clarke, B., and D. R. S. Kirby. 1966. Maintenance of histocompatibility polymorphisms. Nature **211**(999):1000.

Cleaveland, S., M. L. Laurenson, and L. H. Taylor. 2001. Disease of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London **356:**991-999.

Coltman, D. W., J. G. Pilkington, J. A. Smith, and J. M. Pemberton. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution **53:**1259-1267.

Cunningham, A. A. 1996. Disease risks of wildlife translocations. Conservation Biology **10:**349-353.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife – threats to biodiversity and human health. Science **287:**443-449.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica **78:**103-116.

Davies, T. J., and A. B. Pedersen. 2008. Phylogeny and geography predict pathogen community similarity in wild primates and humans. Proceedings of the Royal Society of London **275:**1695-1701.

de Castro, F., and B. Bolker. 2005. Mechanisms of disease induced extinction. Ecology Letters **8:**117-126.

de Groot, N. G., N. Otting, G. G. M. Doxiadis, S. S. Balla-Jhagjhoorsingh, J. L. Heeney, J. J.van Rood, P. Gagneux, and R. E. Bontrop. 2002. Evidence for an ancient selective sweep in the MHC class I gene repertoire of chimpanzees. Proceedings of the National Academy of Sciences **99**(18)**:**11748-11753.

Decamposlima, P. O., R. Gavioli, Q. J. Zhang, L. E. Wallace, R. Dolcetti, M. Rowe, A. B. Rickinson, and M. G. Masucci. 2003. HLA-A11 epitope loss isolates of Epstein-Barr-virus from a highly A11+ population. Science **260:**98-100.

Ditchkoff, S. S., R. L. Lochmiller, R. E. Masters, S. R. Hoofer, and R. A. Van Den Bussche. 2001. Major-histocompatibility-complex-associated variation in secondary sexual traits of White-tailed deer (*odocoileus virginianus*): Evidence for good-genes advertisement. Evolution **55:**616-625.

Doherty, P. C., and R. M. Zinkernagel. 1975. Enhanced immunologic surveillance in mice heterozygous at the *H-2* gene complex. Nature **256:**50-52.

Doron-Faigenboim, A., A. Stern, I. Mayrose, E. Bacharach, and T. Pupko. 2005. Selecton: A server for detecting evolutionary forces at a single amino-acid site. Bioinformatics **21**(9)**:**2101-2103.

Drewe, J. A., M. J. O'Riain, E. Beamish, H. Currie, and S. Parsons. 2012. Survey of infections transmissible between baboons and humans, Cape Town, South Africa. Emerging Infectious Diseases **18**(2)**:**298-301.

Edwards, S., and P. W. Hedrick. 1998. Evolution and ecology of MHC molecules: From genomics to sexual selection. Trends in Ecology and Evolution **13:**305-311.

Egrid, K., and J. L. Brown. 1989. The major histocompatibility complex and female mating preferences in mice. Animal Behavior **38:**548-550.

Ellis, S. A., R. E. Bontrop, D. F. Antczak, K. Ballingall, C. J. Davies, J. Kaufman, L. J. Kennedy, J. Robinson, D. M. Smith, M. J. Stear, R. J. M. Stet, M. J. Waller, L. Walter, and S. G. E. Marsh. 2006. ISAG/IUIS-VIC comparative MHC nomenclature committee report, 2005. Immunogenetics **57:**953-958.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics **34:**487-515.

Findlay, C. S., and J. Houlahan. 1997. Anthropogenic correlates of species richness in southeastern Ontario wetlands. Conservation Biology **11:**1000-1009.

Fischer, J., and D. B. Lindenmayer. 2000. An assessment of the published results of animal relocations. Biological Conservation **96:**1-11.

Formenty, P., C. Boesch, M. Wyers, C. Steiner, F. Donati, F. Dind, F. Walker, and B. Le Guennom. 1999. Ebola virus outbreak among wild chimpanzees living in a rainforest of Cote d'Ivoire. Journal of Infectious Disease **179:**120-126.

Frankham, R., J. D. Ballou, and D. A. Broscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, England.

Frankham, R., and K. Ralls. 1998. Inbreeding leads to extinction. Nature **392:**441-442.

Gibbs, J. P. 1998. Distribution of woodland amphibians along a forest fragmentation gradient. Landscape Ecology **13:**263-268.

Gibbs, J. P. 2001. Demography versus habitat fragmentation as determinants of genetic variation in wild populations. Biological Conservation **100:**15-20.

Gill, T. J. 1983. Immunogenetics of spontaneous abortions in humans. Transplantation **35:**1-6.

Goodall, J. 1988. In the Shadow of Man. Pages 224 Weidenfeld & Nicholson, London, England.

Goossens, B., L. Chikhi, M. F. Jalil, M. Ancrenaz, I. Lackman-Ancrenaz, M. Mohamed, P. Andau, and M. W. Bruford. 2005. Patterns of genetic diversity and migration in increasingly fragmented and declining orangutan (*Pongo pygmaeus*) populations from Sabah, Malaysia. Molecular Ecology **14:**441-456.

Goossens, B., S. M. Funk, C. Vidal, S. Latour, A. Jamart, M. Ancrenaz, E. J. Wickings, C. E. G. Tutin, and M. W. Bruford. 2002. Measuring genetic diversity in translocation programs: Principles and application to a chimpanzee release project. Animal Conservation **5:**225-236.

Groves, C. 2001. Primate Taxonomy. Smithsonian Institution Press, Washington, DC.

Gurd, D. B., T. D. Nudds, and D. H. Rivard. 2001. Conservation of mammals in eastern North American wildlife reserves: How small is too small? Conservation Biology **15:**1355-1363.

Haas, L., H. Hoffer, M. East, P. Wohlsein, B. Liess, and T. Barrett. 1996. Canine distemper virus infection in Serengeti spotted hyenas. Veterinary Microbiology **49:**147-152.

Hall, T. A. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series **41:**95-98.

Hammond, J. A., A. J. Hall, and E. A. Dyrynda. 2005. Comparison of polychlorinated biphenyl (PCB) induced effects on innate immune functions in harbour and grey seals. Aquatic Toxicology **74:**126-138.

Harf, R., and S. Sommer. 2005. Association between major histocompatibility complex class II *DRB* alleles and parasite load in the hairy-footed gerbil, *Gerbillurus paeba*, in the southern Kalahari. Molecular Ecology **14:**85-91.

Hargis, C. D., J. A. Bissonette, and D. L. Turner. 1999. The influence of forest fragmentation and landscape pattern on American martens. Journal of Applied Ecology **36:**157-172.

Harvell, M. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfield, and M. D. Samuel. 2002. Climate warming and disease risk for terrestrial and marine biota. Science **296:**2158-2162.

Havlicek, J., and S. C. Roberts. 2009. MHC-correlated mate choice in humans: A review. Psychoneuroendocrinology **34:**497-512.

Hedrick, P. W. 1994. Evolutionary genetics of the major histocompatibility complex. American Naturalist **143:**945-964.

Hedrick, P. W., and R. Fredrickson. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conservation Genetics **11:**615-626.

Hedrick, P. W., and S. T. Kalinowski. 2000. Inbreeding depression in conservation. Annual Review of Ecology, Evolution, and Systematics **31:**139-162.

Hedrick, P. W., T. J. Kim, and K. M. Parker. 2001a. Parasite resistance and genetic variation in the endangered Gila topminnow. Animal Conservation **4:**103-109.

Hedrick, P. W., K. M. Parker, E. L. Miller, and P. S. Miller. 2001b. Major histocompatibility complex variation in the endangered Przewalski's horse. Genetics **152:**1701-1710.

Hedrick, P. W., and G. Thomson. 1988. Maternal-fetal interactions and the maintenance of HLA polymorphism. Genetics **119:**205-212.

Hill, C. M. 2000. A conflict of interest between people and baboons: Crop raiding in Uganda. International Journal of Primatology **21:**299-315.

Hoffman, M., and C. Hilton-Taylor. 2008. *Papio ursinus*. IUCN. Available from www.iucnredlist.org (accessed 01/25 2012).

Hoffman, T. 2011. The spatial ecology of Chacma baboons (*Papio ursinus*) in the Cape Peninsula, South Africa: Towards improved management and conservation strategies. Ph.D in Zoology edition. University of Cape Town, Cape Town, South Africa.

Hoffman, T. S., and M. J. O'Riain. 2010. The spatial ecology of Chacma baboons (*papio ursinus*) in a human-modified environment. International Journal of Primatology **32:**308-328.

Huchard, E., G. Cowlishaw, M. Raymond, M. Weill, and L. A. Knapp. 2006. Molecular study of MHC-*DRB* in wild Chacma baboons reveals high variability and evidence for transspecies inheritance. Immunogenetics **58:**805-816.

Hughes, A. L., and M. K. Hughes. 1995. Natural selection on the peptide-binding regions of major histocompatibility complex molecules. Immunogenetics **42:**233-243.

Hughes, A. L., and M. Nei. 1988. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature **335:**167-170.

Hughes, A. L., and M. Nei. 1989. Nucleotide substitution at major histocompatibility complex class II loci: Evidence for overdominant selection. Proceedings of the National Academy of Sciences **86:**948-962.

Hughes, A. L., and M. Nei. 1990. Evolutionary relationships of class II major-histocompatibility-complex genes in mammals. Molecular Biology and Evolution 7(6)**:**491-514.

Hughes, A. L., and M. Yeager. 1998. Natural selection at major histocompatibility complex loci of vertebrates. Annual Review of Genetics **32:**415-435.

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate change 2007: synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

IUCN. 1998. IUCN Guidelines for Re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. IUCN, Gland, Switzerland.

Jenkins, P. T., K. Genovese, and H. Ruffler. 2007. Broken screens: the regulation of live animal imports in the United States. Defenders of Wildlife, Washington, DC.

Johnson, N. P. A. S., and J. Mueller. 2002. Updating the accounts: Global mortality of the 1918–1920 "Spanish" influenza pandemic. Bulletin of the History of Medicine **76:**195-115.

Jordan, W. C., and M. W. Bruford. 1998. New perspectives on mate choice and the MHC. Heredity **81:**127-133.

Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Pages 21-132 in H. N. Munro, editor. Mammalian Protein Metabolism. Academic Press, New York, NY.

Kansky, R. and D. Gaynor. 2000. Baboon Management Strategy for the Cape Peninsula. Final Report to the WWF-SA (Table Mountain Fund), Project number ZA 568.

Keller, L., and D. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution **17:**230-241.

Kenter, M., N. Otting, J. Anholts, M. Leuissen, M. Jonker, and R. E. Bontrop. 1992. Evolutionary relationships among the primate MHC-*DQA*1 and *DQA*2 alleles. Immunogenetics **36:**71-78.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University, New York, NY.

Kindt, T. J., B. A. Osborne, R. A. Goldsby, and J. Kuby, editors. 2006. Kuby Immunology. 6th edition. W.H. Freeman and Company, New York, NY.

Knapp, L. A. 2002. Evolution and immunology: Evolutionary anthropology supplement. Evolutionary Anthropology Supplement **1:**140-144.

Knapp, L. A. 2005a. The ABCs of MHC. Evolutionary Anthropology **14:**28-37.

Knapp, L. A. 2005b. Facts, feces and setting standards for the study of MHC genes using noninvasive samples. Molecular Ecology **14:**1597-1599.

Kohn, G. C. 1995. The Wordsworth Encyclopedia of Plague and Pestilence. Facts on File, New York, NY.

Kostyu, D. A., D. V. Dawson, S. Elias, and C. Ober. 1993. Deficit of HLA homozygotes in a Caucasian isolate. Human Immunology **37:**135-142.

Kriger, K. M., F. Peroglou, and J. M. Hero. 2007. Latitudinal variation in the prevalence and intensity of Chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. Conservation Biology **21:**1280-1290.

Lacy, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammology **78:**320-335.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241:**1455- 1460.

Leroy, E. M., P. Rouquet, P. Formenty, S. Souquiere, A. Killbourne, J. M. Froment, M. Bermeljo, S. Smit, W. Karesh, R. Swanepoel, S. R. Zaki, and P. E. Rollin. 2004. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science **303:**387-390.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature **405:**679-681.

Lukas, D., B. J. Bradley, A. M. Nsubuga, D. Doran-Sheehy, M. M. Robbins, and L. Vigilant. 2004. Major histocompatibility complex and microsatellite variation in two populations of wild gorillas. Molecular Ecology **13:**3389-3402.

Lyles, A. M., and A. P. Dobson. 1993. Infectious disease and intensive management: Population dynamics, threatened hosts, and their parasites. Journal of Zoo and Wildlife Medicine **24:**315-326.

Macdonald, I. A. W., and R. M. Cowling. 1996. Guest editorial. Biodiversity and Conservation **5:**525-526.

Markert, J. A., P. R. Grant, B. R. Grant, L. F. Keller, J. L. Coombs, and K. Petren. 2004. Neutral locus heterozygosity, inbreeding and survival in Darwin's ground finches (*Geospiza fortis* and *G. scandens*). Heredity **92:**306-315.

Markow, T., P. W. Hedrick, K. Zuerlein, J. Danilovs, J. Martin, T. Vyvial, and C. Armstrong. 1993. HLA polymorphism in the Havasupai: Evidence for balancing selection. American Journal of Human Genetics **53:**943-952.

McCallum, H., N. Barlow, and J. Hone. 2001. How should pathogen transmission be modeled? Trends in Ecology and Evolution **16**(1295)**:**1300.

McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology and Evolution **10:**190-194.

McClelland, E. E., D. J. Penn, and W. K. Potts. 2003. Major histocompatibility complex heterozygote superiority during coinfection. Infection and Immunity **71:**2079-2086.

McKinney, M. L. 2002. Urbanization, biodiversity, and conservation. Bioscience **52**(10)**:**883-890.

McKinney, M. L. 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation **127:**247-260.

Medzhitov, R., and C. A. Janeway Jr. 1997. Innate immunity: Impact on the adaptive immune response. Current Opinion in Immunology **9:**4-9.

Mellor, P. S., and J. Boorman. 1995. The transmission and geographical spread of African horse sickness and bluetongue viruses. Annals of Tropical Medical Parasitology **89**(1)**:**1-15.

Meltzer, D. G. A. 1993. Historical survey of disease problems in wildlife populations: Southern African mammals. Journal of Zoo and Wildlife Medicine **24**(3)**:**237-244.

Meyers, L. A., and J. J. Bull. 2002. Fighting change with change: Adaptive variation in an uncertain world. Trends in Ecology and Evolution **17:**551-557.

Mitchell, S. E., E. S. Rogers, T. J. Little, and A. F. Read. 2005. Host-parasite and genotype- by environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. Evolution **59:**70-80.

Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature **430:**242-249.

Moritz, C. 1999. Conservation units and translocations: Strategies for conserving evolutionary process. Hereditas **130:**217-228.

Morrell, V. 1999. Are pathogens felling frogs? Science **284:**728-731.

Mwenda, J. M., O. Takenaka, H. Kim, T. Yamamoto, Y. Katsumata, C. S. Bambra, R. Uchihi, and T. Shotake. 1997. Nucleotide sequences of the major histocompatibility complex *DQA*1 locus of Cercopithecus monkeys. Immunogenetics **46:**363-364.

Nei, M., and T. Gojobori. 1986. Simple methods for estimating the numbers of synonymous and non-synonymous nucleotide substitutions. Molecular Biology and Evolution **3:**418-426.

Nei, M., X. Gu, and T. Sitnikova. 1997. Evolution by the birth-and-death process in multigene families of the vertebrate immune system. Proceedings of the National Academy of Sciences **94:**7799-7806.

Nsubuga, A.M., M.M. Robbins, A.D. Roeder, P.A. Morin, C. Boesch, and L. Vigilant. 2004. Factors affecting the amount of genomic DNA extracted from ape feces and the identification of an improved sample storage method. Molecular Ecology. **13**:2089-2094.

O'Keefe, K. J., and J. Antonovics. 2002. Playing by different rules: The evolution of virulence in sterilizing pathogens. American Naturalist **159:**597-605.

Paterson, S., and J. M. Pemberton. 1997. No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. Proceedings of the Royal Society of London **264:**1813-1819.

Pedersen, A. B., K. E. Jones, C. L. Nunn, and S. A. Altizer. 2007. Infectious disease and mammalian extinction risk. Conservation Biology **21:**1269-1279.

Penn, D., and W. K. Potts. 1999. The evolution of mating preferences and major histocompatibility genes. American Naturalist **153:**145-164.

Penn, D. J., K. Damjanovich, and W. K. Potts. 2002. MHC heterozygosity confers a selective advantage against multi-strain infections. Proceedings of the National Academy of Sciences **99:**11260-11264.

Piertney, S. B., and M. K. Oliver. 2006. The evolutionary ecology of the major histocompatibility complex. Heredity **96:**7-21.

Pimm, S. L., L. Dollar, and O. L. Bass Jr. 2006. The genetic rescue of the Florida panther. Animal Conservation **9:**115-122.

Pimm, S. L., J. R. Gareth, J. L. Gittleman, and T. M. Brooks. 1995. The future of Biodiversity. Science **269**(5222)**:**347-350.

Pimm, S. L., and P. Raven. 2000. Extinction by numbers. Nature **403:**843-845.

Potts, W. K., C. J. Manning, and E. K. Wakeland. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature **352:**619-621.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature **439:**161-167.

Ravasi, D. 2009. Gastrointestinal parasite infections in Chacma Baboons (*Papio ursinus*) of the Cape Peninsula, South Africa: The influence of individual, troop, and anthropogenic factors. PhD in Zoology. University of Cape Town, Cape Town, South Africa.

Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology **17**(1)**:**230-237.

Renquist, D. M., and R. A. Whitney. 1987. Zoonoses acquired from pet primates. Veterinary Clinics of North America: Small Animal Practice **17**(1)**:**219-240.

Rouget, M., D. M. Richardson, R. M. Cowling, J. W. Lloyd, and A. T. Lombard. 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the cape floristic region, South Africa. Biological Conservation **112:**63-85.

Rozas, J., P. Librado, and J. C. Sanchez-DelBarrio. 2010. DNAsp v 5.10.01. Universitat de Barcelona, Barcelona, Spain.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4:**406-425.

Sauermann, U., P. Nurnberg, F. Bercovitch, J. Berard, A. Trefilov, A. Widdig, M. Kessler, J. Schmidtke, and M. Krawczak. 2001. Increased reproductive success of MHC class II heterozygous males among free-ranging Rhesus macaques. Human Genetics **108:**249-254.

Schad, J., J. U. Ganzhorn, and S. Sommer. 2005. MHC constitution and parasite burden in the Malagasy mouse lemur, *Microcebus murinus*. Evolution **59:**439-450.

Schad, J., S. Sommer, and J. U. Ganzhorn. 2004. MHC variability of a small lemur in the littoral forest fragments of southeastern Madagascar. Conservation Genetics **5:**299-309.

Scharf, S. J., G. T. Horn, and H. A. Erlich. 1986. Direct cloning and sequence analysis of enzymatically amplified genomic sequences. Science **233**(4768)**:**1076-1078.

Schrag, S. J., and P. Wiener. 1995. Emerging infectious disease: What are the relative roles of ecology and evolution? Trends in Ecology and Evolution **10**(8)**:**319-324.

Scott, M. E. 1988. The impact of infection and disease on animal populations: Implications for conservation biology. Conservation Biology **2:**40-56.

Sher, A., S. Hieny, and S. James. 1984. Mechanisms of protective immunity against *S. mansoni* infection in mice vaccinated with irradiated cercariae: VI. influence of the major histocompatibility complex. Parasite Immunology **6:**319-328.

Shriner, D., D. C. Nickle, M. A. Jensen, and J. I. Mullins. 2003. Potential impact of recombination on sitewise approaches for detecting positive natural selection. Genetics Research **81**(2)**:**115-121.

Smith, K. F., K. Acevedo-Whitehouse, and A. B. Pedersen. 2009. The role of infectious diseases in biological conservation. Animal Conservation **12:**1-12.

Sommer, S. 2005. The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Frontiers in Zoology **2**(16)**:**1-18.

Soorae, P. S., and L. R. Baker, editors. 2002. Re-introduction NEWS: Special Primate Issue. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE.

Spielman, D., B. W. Brook, D. A. Briscoe, and R. Frankham. 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? Conservation Genetics **5:**439-448.

Steffan-Dewenter, I., U. Muzenberg, C. Burger, C. Thies, and T. Tscharntke. 2002. Scale dependent effects of landscape context on three pollinator guilds. Ecology **83:**1421-1432.

Stern, A., A. Doron-Faigenboim, E. Erez, E. Martz, E. Bacharach, and T. Pupko. 2007. Advanced models for detecting positive and purifying selection using a Bayesian inference approach. Nucleic Acids Research **35:**W506-W511.

Strum, S. C. 1994. Prospects for management of primate pests. Revue d'Ecologie (La Terre et la Vie) **49:**295-306.

Strum, S. C. 2005. Measuring success in primate translocation: A baboon case study. American Journal of Primatology **65:**117-140.

Takahata, N., and M. Nei. 1990. Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. Genetics **124:**967-978.

Tallmon, D. A., G. Luikart, and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution **19**(9)**:**489-496.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution **28**(10)**:** 2731-2739.

Templeton, A. R., K. Shaw, E. Routman, and S. K. Davis. 1990. The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden **77:**13-27.

Thomas, M. L., J. H. Harger, D. K. Wagener, B. S. Rabin, and T. J. Gill. 1985. HLA sharing and spontaneous abortion in humans. American journal of Obstetrics and Gynecology **151**(1053)**:**1058.

Thompson, J. D., D. G. Higgins, and T. J. Gibbons. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research **22:**4673-4680.

Thursz, M. R., D. Kwiatkowski, C. E. Allsopp, B. M. Greenwood, H. C. Thomas, and A. V. S. Hill. 1995. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. New England Journal of Medicine **332:**1065-1069.

Tigas, L. A., D. H. Van Vuren, and R. M. Sauvajot. 2002. Behavioral responses of bobcat and coyotes to habitat fragmentation and corridors in an urban environment. Biological Conservation **108:**299-306.

Torchin, M. E., and C. E. Mitchell. 2004. Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment **2:**183-190.

van Doorn, A. 2009. The interface between socioecology and management of Chacma baboons (*Papio ursinus*) in the Cape Peninsula, South Africa. Ph.D edition. University of Cape Town, Cape Town, South Africa.

van Tienderen, P. H., A. A. de Haan, G. van der Linden, and B. Vosman. 2002. Biodiversity assessment using markers for ecologically important traits. Trends in Ecology and Evolution **17:**577-582.

Vernier, L., and L. Fahrig. 1996. Habitat availability causes the species abundance-distribution relationship. Oikos **76:**564-570.

Wedekind, C., and S. Furi. 1997. Body odor preferences in men and women: Do they aim for specific MHC combinations or simply heterozygosity? Proceedings of the Royal Society of London **264:**1471-1479.

Wedekind, C., T. Seebeck, F. Bettens, and A. J. Paepke. 1995. MHC-dependent mate preferences in humans. Proceedings of the Royal Society of London **260:**245-249.

Wenink, P. W., A. F. Groen, M. E. Roelke-Parker, and H. H. T. Prins. 1998. African buffalo maintain high genetic diversity in the major histocompatibility complex in spite of historically known population bottlenecks. Molecular Ecology **7:**1315-1322.

Westerdahl, H. 2004. No evidence of an MHC-based female mating preference in Great Reed warblers. Molecular Ecology **13:**2465-2470.

Wettstein, W., and B. Schmid. 1999. Conservation of arthropod diversity in montane wetlands: Effect of altitude, habitat quality and habitat fragmentation on butterflies and grasshoppers. Journal of Applied Ecology **36:**363-373.

Williams, E. S., E. T. Thorne, M. J. Appel, and D. W. Belitsky. 1988. Canine distemper in Black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases **24:**385-398.

Wilson, E. O. 1992. The diversity of life. W.W. Norton and Company, New York, NY.

With, K. A., and T. O. Crist. 1995. Critical thresholds in species' responses to landscape structure. Ecology **76:**2446-2459.

Woolhouse, M. E. J., and S. Gowtage-Seueria. 2005. Host range and emerging and reemerging pathogens. Emerging Infectious Diseases **11:**118-1847.

Woolhouse, M. E. J., D. T. Haydon, and R. Anita. 2005. Emerging pathogens: The epidemiology and evolution of species jumps. Trends in Ecology and Evolution **20:**238-244.

Yamakazi, K., E. A. Boyse, V. Mike, H. T. Thaler, B. J. Mathieson, J. Abbott, J. Boyse, Z. A. Zayas, and L. Thomas. 1976. Control of mating preference in mice by genes in the major histocompatibility complex. Journal of Experimental Medicine **144:**1324-1335.

Yang, Z., R. Nielsen, N. Goldman, and A. M. Pedersen. 2000. Codon-substitution models for heterogeneous selection pressure at amino acid sites. Genetics **155:**431-449.

Appendix 1: Frequency of unique Papio ursinus MHC-*DQA*1 alleles per sampling site

	Betty's Bay	Buffelsbay	Cape Point	Slangkop	Tokai	Da Gama	Groot Olifantbos	Heidelberg	Pringle Bay	Rooiels
Paur_*DQA*1*01	0.56	0.85	0.87	0.93	0.76	0.43	0.92	0.50	0.76	0.67
Paur_*DQA*1*02	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*03	-	-	0.03	-	0.03	-	-	-	-	-
Paur_*DQA*1*04	-	-	0.03	-	-	-	-	-	-	-
Paur_*DQA*1*05	-	0.08	0.03	-	-	-	-	-	-	-
Paur_*DQA*1*06	-	-	-	-	-	0.04	-	-	-	-
Paur_*DQA*1*07	-	-	0.03	-	-	-	-	-	-	-
Paur_*DQA*1*08	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*09	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*10	-	-	-	-	-	-	-	-	0.06	-
Paur_*DQA*1*11	-	-	-	0.05	-	-	-	-	-	-
Paur_*DQA*1*12	-	-	-	-	-	-	-	0.05	-	-
Paur_*DQA*1*13	-	-	-	-	-	-	-	-	0.06	-
Paur_*DQA*1*14	-	-	-	0.03	-	-	-	-	-	-
Paur_*DQA*1*15	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*16	-	-	-	-	-	-	-	-	0.06	-
Paur_*DQA*1*17	-	-	-	-	-	-	-	0.05	-	-
Paur_*DQA*1*18	-	-	0.03	-	-	-	-	-	-	-
Paur_*DQA*1*19	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*20	-	0.08	-	-	-	-	-	-	-	-
Paur_*DQA*1*21	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*22	-	-	-	-	-	-	0.04	-	-	-
Paur_*DQA*1*23	-	-	-	-	-	-	-	-	-	0.03
Paur_*DQA*1*24	-	-	-	-	-	-	-	0.05	-	-
Paur_*DQA*1*25	-	-	-	-	0.08	-	0.04	-	-	-
Paur_*DQA*1*26	-	-	-	-	0.14	0.35	-	0.10	0.06	0.08
Paur_*DQA*1*27	-	-	-	-	-	-	-	0.05	-	-
Paur_*DQA*1*28	0.44	-	-	-	-	0.13	-	0.15	-	0.06
Paur_*DQA*1*29	-	-	-	-	-	0.04	-	-	-	-
Paur_*DQA*1*30	-	-	-	-	-	-	-	0.05	-	-