GENETIC MANAGEMENT OF THE BABOON POPULATION IN THE SUIKERBOSRAND NATURE RESERVE

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbols

°C	degrees Celsius
%	percent
µ	micro: 10^{-6}
n	nano: 10^{-9}
p	pico: 10^{-12}
&	and
∞	infinity
®	registered trademark
™	trademark

Abbreviations

A_{260}/A_{280}	ratio of absorbency measured at 260 nm and 280 nm
AFLP	amplified fragment length polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
AU	absorbance units
bp	base pair
CITES	Convention on International Trade in Endangered Species
cm	centimeter
CTD	cellular telemetry device
ddH_2O	double distilled water
df	degrees of freedom
DNA	deoxyribonucleic acid
DNS	deoksiribonukleïensuur
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediamine tetra-acetic acid: $C_{10}H_{16}N_2O_8$
et al.	*et alii*: and others
etc.	*et cetera*: and so on
ETOH	ethanol: CH_3CH_2OH
F	forward (primer)

g	gram
GIMLET	Genetic Identification with Multilocus Tags
GIS	geographic information system
ha	hectare
Ho	observed heterozygosity
Hz	unbiased heterozygosity
ID	identification
i.e.	*id est*: in other words, that is
K	clusters
km	kilometer
µl	microliter
µM	micromolar
M	molarity
Mg^{2+}	magnesium ion
$MgCl_2$	magnesium chloride
min	minute
ml	milliliter
mM	millimolar
mm	millimeter
ng	nanogram
nm	nanometer
No.	number
OMU	one male unit
PCR	polymerase chain reaction
PI	probability of identity
pmol	pico mol
R	reverse (primer)
RFLP	restriction fragment length polymorphism
rfu	relative fluorescent unit
s	seconds
SD	standard deviation
STR	short tandem repeat
T_a	annealing temperature
T_m	melting temperature
U	unit

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CHAPTER ONE

Introduction

Conservation genetics is a field that makes use of molecular genetic techniques in order to answer questions related to ecology, behavior, social structure and conservation of a population (Matsui *et al*., 2007; Vigilant & Guschanski, 2009). In the last decade, several molecular techniques have been applied in conservation genetics. These methods include deoxyribonucleic acid (DNA) fingerprinting, sequencing of mitochondrial DNA, restriction fragment length polymorphism (RFLP) analysis, the sequencing of genomes, genotyping of loci using microsatellite markers and amplified fragment length polymorphisms (AFLPs) (Aitken *et al*., 2004).

In earlier years, genetic issues were not considered important in the practical management of wild populations in natural habitats in South Africa. However, there has been an increased emphasis on managing the genetic aspects of non-human primate populations due to advances in molecular techniques (Williams-Blangero *et al*., 2002). The use of human microsatellite markers on primate populations can provide valuable insights as part of population studies (Newman *et al*., 2002).

Management of the chacma baboon (*Papio ursinus* (Kerr, 1792)) population in the Suikerbosrand Nature Reserve has taken a low priority due to insufficient data to support management decisions. This deficiency in data is a result of the nature of baboons and the difficulty experienced to obtain data. Certain aspects of the population cannot be determined through observation alone. The viability of a population as well as its evolutionary potential is determined by factors such as genetic variation, breeding system, effective population size, gene flow and the genetic distance of nearby populations (Morin *et al*., 1993). Thus it is essential to quantify these genetic relationships. The dispersal of individuals and gene flow between troops is vital as it has an effect on the overall genetic structure of the population (Vigilant & Guschanski,

2009). If the adequate level of gene flow is not maintained, the population could potentially face the risk of inbreeding, leading to a loss of genetic diversity. Conservation biologists need to understand genetic diversity before conservation programs can be designed to manage a population. Genetic diversity is maintained through natural processes such as mutation, migration and selection. Mutation and migration adds to variation whereas directional selection removes variation. The population size has an important impact on the balance between these factors. Smaller populations will generally show lower levels of genetic diversity compared to a larger population (Frankham *et al*., 2002). The processes of selection, genetic drift, mutation and migration determine the level of genetic diversity (Frankham *et al*., 2002). Furthermore, by compiling genetic profiles of the individuals in the population, insights can be provided on aspects such as mating systems, paternity, relatedness and dispersal and migration patterns. Through the collection of fecal samples from sleeping sites, individual profiles could potentially be used to non-invasively determine the number of individuals in the population. Genetic markers can also be used to identify populations of concern where genetic factors are prone to affect the long term survival of the population (Frankham *et al*., 2002).

The principles mentioned are applied in this population study to provide insights on the chacma baboon population in the Suikerbosrand Nature Reserve. Throughout this dissertation, a population will be defined as individuals in the same geographical region (Evanno *et al*., 2005). A population will thus consist of a number of different troops. The main part of this dissertation is presented as three independent but interlinked sections. In Chapter 2, the population structure of the Suikerbosrand baboon population was determined. The objectives include compiling individual profiles of all the sampled individuals, quantification of genetic relationships and determining levels of gene flow between the troops. Chapter 3 entails the optimization of the collection method for fecal samples in order to ensure high DNA quality when collecting fecal samples from a sleeping site at Suikerbosrand. In Chapter 4, the results of Chapter 3 were applied to investigate the use of non-invasive sampling as a method to identify individuals from a

single sleeping site and thus determining the number of individuals in a troop. Chapter 5 is a short discussion of knowledge gathered from this study.

CHAPTER TWO

Population structure study

2.1 Introduction

The highly adaptable nature of primates and their ability to change their behavior according to what they learn makes them very successful crop raiders and may potentially lead to human/non-human primate conflict (Hill, 2000). Baboons are the species most often at the centre of such controversies in South Africa.

2.1.1 Background on chacma baboons

Chacma baboons of the genus *Papio* are distributed throughout the southern African subregion, but in the Nama-Karoo and Succulent Karoo biomes they are only found where conditions are suitable (Skinner & Chimimba, 2005). The ability of baboons to dig for food makes it possible to obtain food in areas where resources above the ground are scarce. This ability enables them to inhabit areas from which other primates might be excluded (Barrett & Henzi, 2008).

The size of the troop's home range is influenced by the number of individuals in the troop as well as the availability of water. The home ranges of different troops may overlap and troops may have more than one sleeping site within a home range. Each sleeping site is urine-stained, marked by the accumulation of dung, and has a distinctive smell. A troop may use the same sleeping site for a longer period or use different sites on a rotational basis (Skinner & Chimimba, 2005).

Chacma baboons generally live in large multi-male:multi-female troops that may number up to 130 individuals in exceptional cases. On average, troop size range between 20 to 80 individuals (Barrett & Henzi, 2008). The food resources in a habitat largely influence the

troop size. Habitats with richer food resources will support larger troops. Troop numbers vary through births, deaths, migration of males, etc. (Skinner & Chimimba, 2005). If a troop gets too large for the amount of food resources available, it will split into smaller groups. The troop usually splits along lines of genetic relatedness or close friendships, and it is usually based on the females' choice. This can result in groups known as OMU's or one-male units. The formation of OMU's usually results in certain behavioral differences. These differences can be seen in foraging patterns, sexual behaviors, inter-group relations, intra-group relations and the general behavior patterns of males (Hamilton III & Bulger, 1992).

The females are philopatric and remain in their natal troop, but the males relocate between troops as soon as they reach adulthood (Skinner & Chimimba, 2005; Barrett & Henzi, 2008). In chacma baboons, the alpha-male can monopolize matings resulting in the majority of infants having the same father during his tenure (Barrett & Henzi, 2008).

2.1.2 The chacma baboon population of the Suikerbosrand Nature Reserve

Suikerbosrand Nature Reserve is located approximately 50 km south from Johannesburg near the town Heidelberg. The size of the reserve is 16500 ha. The biomes present on Suikerbosrand are the Moist Cool Highveld Grassland and the Rocky Highveld Grassland (Bredenkamp & Van Rooyen, 1996).

There is little information on the spatial distribution and population size of the chacma baboons in Suikerbosrand. Four different counts have been undertaken since the proclamation of the reserve in 1974 but only two of the counts managed to provide an estimate of the total number of the population (Hennop, 2007).

From the limited available information, it is thought that the size of the population increased from an estimated 350 baboons in 1981 to the current estimated population of between 611 and 764 animals. However, it is not clear whether this increase is a result of

improved census methods, pressure as a result of persecution and baboons seeking a safe haven within reserve borders, or better food availability in recent times.

Increased anthropogenic developments around the reserve are a great concern. Habitat outside the reserve was previously utilized by the baboons, but is becoming inaccessible as a result of the developments and is no longer available for foraging. As a result, crop raiding and damage to neighboring land is a significant problem. Agricultural holdings, commercial farm land and residents within the town limits of Heidelberg are all affected. Raiding is less common in residential areas, but old single males may occasionally raid fruit trees in these areas. In these cases the animal is captured by Suikerbosrand personnel and brought back to the reserve. Culling of the animals that cause trouble has been considered. However, the status of the population must be determined before such serious intervention can take place. Data on the whereabouts of the troops are particularly important as culling of these groups might increase the problem as a result of negative effects on the population's social behavior (Johnny Hennop, Suikerbosrand Nature Reserve, personal communication).

A genetic analysis study was therefore conducted in order to reach the objectives of Suikerbosrand Nature Reserve to collect baseline spatial and population data in order to make informed management decisions.

2.1.3 Genetic management of a population

Field studies are necessary to identify changes in the ecosystem and natural populations, and to identify factors that influence the viability of individuals or the population. These aspects are important in order to make management decisions for a declining population or to monitor animals that have been relocated or reintroduced (Young & Isbell, 1994).

Four key parameters are estimated when studying the persistence of a species in its environment. These parameters include birth and death rates, and emigration and immigration rates (Hanski, 2001). Direct observation is usually applied to determine birth

and death rates. Even though emigration and immigration can also be determined through observation, the use of genetic markers to determine gene flow is an important approach because it can aid in the understanding of dispersal patterns. The accuracy of the results obtained from the application of genetic markers depends on the level of genetic differentiation in a population as well as the number of loci used (Berry *et al*., 2004). DNA analysis of a social population such as baboons can clarify a number of uncertainties with regard to the population structure, dispersal patterns and relatedness. The genetic structure of populations, and genetic connectivity among populations, can also be determined through DNA analysis.

Vigilant & Guschanski (2009) defined genetic structure as "the presence of a detectable pattern of genetic subdivision within a sampled population". Various features add to this structure such as population history, demography, social structure, aspects with regard to dispersal, and habitat. One of the most important aspects of an organism's life history is the dispersal of individuals from the natal group with the aim of breeding (Handley & Perrin, 2007). In general, there are significant differences between the sexes with regard to the distance traveled and the rate of dispersal (Handley & Perrin, 2007). Patterns of relatedness among individuals in a social group are influenced by sex-biased dispersal and male reproductive success (Altmann *et al*., 1996; De Ruiter & Geffen, 1998). Males are generally the dispersing sex in mammals. Dispersal occurs in order to avoid inbreeding and as a result of factors such as competition for resources and competition for mates (Handley & Perrin, 2007). In order to completely understand dispersal patterns and make conjectures thereof, a combination of field observations as well as genetic analyses is required. Data collected through observations enhance our understanding of species behavior and social structure, whereas genetic analyses allow quantification of how dispersal translates into gene flow (Handley & Perrin, 2007). Gene flow is the result of the dispersal of individuals within and between populations. However, the dispersing individual must reproduce effectively in the location before gene flow can occur (Whitlock & McCauley, 1999). The genetic structure of a population is determined by the dispersal patterns and therefore it is a very important aspect of genetic analysis (Vigilant & Guschanski, 2009). Knowledge on the level of gene flow within and among

populations can be used to determine whether translocation of individuals is necessary in order to maintain genetic diversity and prevent inbreeding (Frankham *et al*., 2002).

The probability that an individual is homozygous at a locus is increased by inbreeding. Naturally outbreeding populations contain low frequencies of deleterious alleles. These deleterious alleles are mostly partially recessive and inbreeding increases the risk of expressing it as homozygotes (Frankham *et al*., 2002). The level of homozygosity is increased on a genome-wide level when inbreeding is present in a population. As a result, the fitness of a population might be reduced (Hansson & Westerberg, 2002).

Genetic diversity is a characteristic of individuals as well as populations (Lacy, 1997). Within individuals, diversity is generally known as the percentage of loci at which an individual is heterozygous (Lacy, 1997). In a population, diversity is measured by the gene diversity, the number of alleles per locus or the percentage of loci that are polymorphic (Nei, 1973; Lacy, 1997). Genetic diversity within a population allows it to evolve in reaction to environmental changes such as diseases, pests, parasites, etc. The conservation thereof is a fundamental concern in conservation biology. Mutations, genetic drift and natural selection determines the level of diversity present in a population. Mutations generate genetic diversity whereas genetic drift reduces it. Natural selection may either decrease genetic diversity as a result of the fixation of alleles or maintain it (Frankham, 1996). Correlation exists between the level of heterozygosity and the size of a population (Reed & Frankham, 2003).

2.1.4 Previous studies done on primates

Certain aspects regarding the social structure of primates are difficult to address through observational studies alone. The field of molecular ecology can aid in the understanding of these aspects (Di Fiore, 2005).

Previous studies have ascertained similarities between the chromosomes of humans and baboons (Cox *et al*., 2006). Many microsatellite loci identified in humans are conserved

across primate taxa and amplify in non-human primates (Newman *et al*., 2002). As a result, microsatellite markers developed for humans have been used successfully in the genetic analysis of several primate species.

DNA fingerprinting techniques were used by Bruford *et al*. (1993) to determine reproductive success in a captive population of guinea baboons in Brookfield Zoo. Parentage (Smith *et al*., 1999) and patterns of hybridization (Tung *et al*., 2008) has also been common research topics with regard to baboon populations.

Chimpanzees in Gombe National Park have been studied making use of non-invasive sampling to determine parentage (Morin *et al*., 1993; Constable *et al*., 2001), community structure, phyleography (Morin *et al*., 1993), and relatedness (Inoue *et al*., 2008).

Genetic diversity as part of the overall genetic structure of a population is one of the most important aspects of conservation genetics and has been studied in rhesus monkeys (Andrade *et al*., 2004), bonobos (Eriksson *et al*., 2004), owl monkeys (Lau *et al*., 2004), grey mouse lemurs (Fredsted *et al*., 2005), capuchin monkeys (Amaral *et al*., 2005) and mandrills (Charpentier *et al*., 2005).

Elucidating relatedness is important especially for populations with low genetic diversity or for populations that forms part of breeding programs. Studies to determine relatedness have been conducted on long-tailed macaques (De Ruiter & Geffen, 1998), orangutans (Immel *et al*., 1999), vervet monkeys (Newman *et al*., 2002) and white-faced capuchin monkeys (Muniz & Vigilant, 2008).

The effect of landscape features (Liu *et al*., 2009) and habitat fragmentation (Milton *et al*., 2009) on the population genetic structure was studied on Yunnan snub-nosed monkeys and howler monkeys respectively.

Over the past ten years molecular techniques have been applied to provide insight on several primate populations from different habitats across the world. These techniques

can also be applied to answer questions with regard to the chacma baboon population in the Suikerbosrand Nature Reserve.

2.1.5 Aim of the population structure component

The aim of this study is to conduct a genetic analysis study using cross-species microsatellite markers on baboons living in different social groups in the Suikerbosrand Nature Reserve.

2.1.6 Objectives

The objective of this study is to apply genetic management as a credible tool for the conservation of baboons in the Suikerbosrand Nature Reserve that will include:

- Individual identification
- Determining genetic relationships between the different troops
- Determining levels of gene flow among troops
- Estimates of variability within troops
- Construction of a genetic database

2.2 Material and Methods

2.2.1 Study population

Suikerbosrand Nature Reserve currently has an estimated chacma baboon population of between 600-700 animals consisting of 15 troops. The average troop size is estimated to be between 30 to 40 animals (Johnny Hennop, Suikerbosrand Nature Reserve, personal communication). The study population included 145 chacma baboons from 14 troops.

The Suikerbosrand field team used Cellular Telemetry Devices (CTD's) for the collection of spatial data in order to identify and track the troops. The sites for trapping of the troops

were selected close to known sleeping sites. Females were mostly collared with the CTD's as the males transfer between troops (Hennop, 2007).

2.2.2 Sample collection

Samples were collected from 14 of the 15 troops. Samples could not be collected from the last troop due to difficulty experienced in capturing this troop. Figure 2.1 shows the fourteen sites where samples were successfully collected from the different troops.

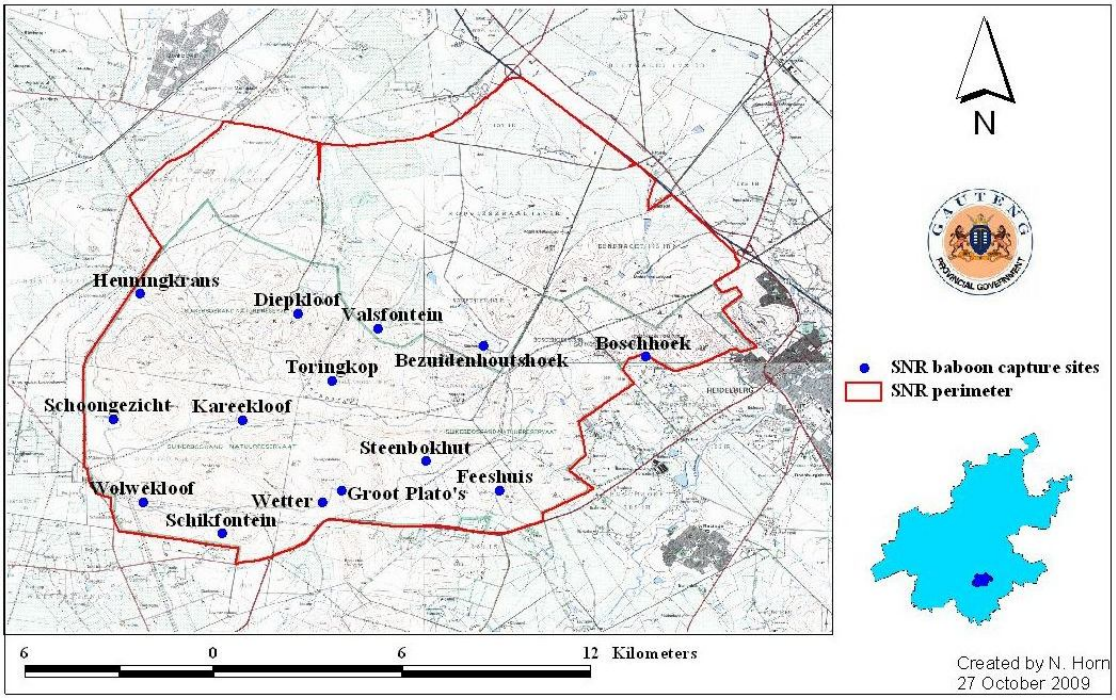

Figure 2.1: Capture sites on Suikerbosrand Nature Reserve (Created by Natalie Horn, SNR)

Research permits were obtained from the Gauteng Directorate of Nature Conservation Permits Office to collect the samples from Suikerbosrand Nature Reserve (Permit No. CPF6-1293; CPF6-1336) and export the samples from the Gauteng Province (Permit No. CPC2-0727; CPC2-0938; CPC2-1400). A permit was also obtained from the Department of Tourism, Environmental and Economic Affairs of the Free State Province for the import of the samples into the Free State (Permit No. HK/P1/08501/001).

A unique collar identification (ID) number was assigned to each sampled individual by the Suikerbosrand field team. Upon arrival at the laboratory, a laboratory number was allocated to each sample according to the date the sample was received. Each collar ID number was recorded with the assigned laboratory number (See Appendix A).

Blood, hair and tissue samples were collected from all the captured animals. Blood samples were collected in ethylenediamine tetra-acetic acid (EDTA) tubes, with the exception of twelve animals from which blood could not be drawn. An ear notch of approximately 5-10 millimeter (mm) was taken as an additional sample source and it was used for DNA extraction in the few cases where no blood sample was available. The blood and tissue samples were stored at -20 degrees Celsius (˚C) until required for DNA extraction. Approximately 50 hairs with roots was taken from each animal and stored in plastic bags at room temperature. A total of 145 samples were collected.

The number of samples collected from each troop is listed in Table 2.1; however, samples could not be obtained from all the individuals. Noser & Byrne (2007) suggest that baboons can form a mental representation of important locations (such as capturing sites) and recognize alternative routes to get to a certain point. They further imply that baboons are tied to a network of learned sequences of landmarks. As a result, after a certain period of time, the baboon troops might recognize the capturing sites and deliberately avoid it. This could explain the last troop avoiding the capturing site that was set up along the troop's usual route. The highest number of samples collected was from the Boschhoek troop (15) and the lowest number of samples was collected from the Schikfontein troop (4).

Five additional samples were collected from a small chacma baboon troop in the Johannesburg Zoo in order to have a control outgroup for comparison.

Table 2.1: Number of samples collected for each troop at Suikerbosrand.

TROOP	NO. OF ANIMALS SAMPLED
Bezuidenhoutshoek	11
Boschhoek	15
Diepkloof	13
Feeshuis	14
Groot Plato's	7
Heuningkrans	13
Kareekloof	14
Schikfontein	4
Schoongezicht	12
Steenbokhut	11
Toringkop	6
Valsfontein	6
Wetter	6
Wolwekloof	12
Average no. of samples per troop	**10.28**

(Refer to Appendix A for a complete list of samples collected.)

2.2.3 DNA extraction

The source from which DNA was extracted for all the samples are indicated in Appendix A. DNA extraction of blood and tissue samples was performed using the QIAGEN QIAamp®[1] DNA Mini Kit. Genomic DNA was extracted from whole blood samples following the *Blood and Body Fluid Spin Protocol*. The tissue samples were extracted following the *Tissue Protocol*. The QIAGEN QIAamp® DNA Micro Kit was used for the extraction of the hair samples, following the protocol for *Isolation of Genomic DNA from Forensic Case Work Samples*. The QIAGEN QIAamp® DNA Micro Kit is used in forensic applications to extract DNA from samples with potentially low amounts of DNA. The manufacturer's instructions were followed for all the extractions.

[1] QIAamp is a registered trademark of QIAGEN GmbH, Hilden, Germany

2.2.4 Determining DNA concentration

DNA yield is determined from the concentration of DNA in the eluate. The concentration was determined with a NanoDrop ND1000 spectrophotometer by measuring absorbance at 260 nanometer (nm). The calculated absorbance is correlated with concentration using the Beer-Lambert equation (Equation 2.1).

Equation 2.1: Beer-Lambert equation

$$A = E \times b \times c$$

A = Absorbance represented in absorbance units (AU)

E = wavelength-dependant molar absorptivity coefficient (extinction coefficient) with units of liter/mol-cm

b = path length in cm

c = analyte concentration in moles/liter or molarity (M)

The Beer-Lambert equation is modified for the quantification of nucleic acids. The manipulated equation is given below.

Equation 2.2: Manipulated equation derived from the Beer-Lambert equation

$$c = (A \times e)/b$$

c = nucleic acid concentration in ng/µl

A = absorbance in AU

e = wavelength dependant extinction coefficient in ng-cm/µl

b = path length in cm

2.2.5 Primers

Microsatellite loci or short tandem repeats (STR's) are widely distributed through the genome of eukaryotes (Tautz & Renz, 1984). The use of microsatellites has become extremely prevalent since the mid 1990's. Microsatellite markers show high polymorphism and are non-coding repetitive DNA regions consisting of tandem repeats

of 2 to 6 nucleotides. The most common choices for microsatellites in molecular genetic studies is dinucleotide, trinucleotide and tetranucleotide repeats (Selkoe & Toonen, 2006).

The DNA surrounding the microsatellite locus is known as the flanking region. Primers can be designed to bind to the flanking region and amplify a microsatellite locus by using PCR (Selkoe & Toonen, 2006). The development of new primers is comparatively costly and time consuming. With the application of cross-species markers, these limitations can be overcome (Coote & Bruford, 1996). Cross-species markers are molecular markers that are developed in one species but can be used in other species of the same family. Human-specific microsatellite markers have become a powerful tool and are widely applied in primate research.

A panel of 11 human microsatellite markers was selected based on results of previously published articles of studies done on primates. The selected loci had to be, preferably, tetranucleotide repeats in order to avoid stutter bands and improve resolution, and polymorphic. The primers used for this study are listed in Table 2.2. The sequences of the primers are indicated in Table 2.3.

Table 2.2: Human microsatellite markers selected for this study

Locus	GenBank Reference	Cross-species Reference	Species	Variability
D1S518	G07854	Newman *et al.* (2002)	*Chlorocebus aethiops*	Polymorphic
		Grobler *et al*. (2006)	*Chlorocebus aethiops*	Not indicated
D2S1326	G08136	Bradley *et al.* (2000)	*Pan troglodytes*	Polymorphic
			Gorilla gorilla	Polymorphic
		Constable *et al.* (2001)	*Pan troglodytes*	Polymorphic
		Bergl & Vigilant (2007)	*Gorilla gorilla*	Polymorphic
		Liu *et al.* (2008)	*Rhinopithecus bieti*	Polymorphic
D3S1768	G08287	Smith *et al*. (1999)	*Papio hamadryas*	Polymorphic
		Bayes *et al.* (2000)	*Papio anubis*	Polymorphic
		Andrade *et al*. (2004)	*Macaca mulatta*	Polymorphic
		Kanthaswamy *et al*. (2006)	*Macaca mulatta*	Polymorphic
		Liu *et al.* (2008)	*Rhinopithecus bieti*	*Not indicated*
D4S243	M87736	Smith *et al*. (1999)	*Papio hamadryas*	Polymorphic
		Bayes *et al.* (2000)	*Papio anubis*	Polymorphic
		Constable *et al.* (2001)	*Pan troglodytes*	Polymorphic
		Newman *et al.* (2002)	*Chlorocebus aethiops*	Polymorphic
		Liu *et al.* (2008)	*Rhinopithecus bieti*	*Not indicated*
D5S1457	G08431	Smith *et al*. (1999)	*Papio hamadryas*	Polymorphic
		Bayes *et al.* (2000)	*Papio anubis*	Polymorphic
		Goossens *et al*. (2000)	*Pongo pygmaeus*	*Not indicated*
		Kanthaswamy *et al*. (2006)	*Macaca mulatta*	Polymorphic
		Bergl & Vigilant (2007)	*Gorilla gorilla*	Polymorphic
		Jeffery *et al.* (2007)	*Gorilla gorilla*	*Not indicated*
		Liu *et al.* (2008)	*Rhinopithecus bieti*	Polymorphic
D7S2204	G08635	Bradley *et al.* (2000)	*Pan troglodytes*	Polymorphic
			Gorilla gorilla	Polymorphic
		Bergl & Vigilant (2007)	*Gorilla gorilla*	Polymorphic
		Liu *et al.* (2008)	*Rhinopithecus bieti*	Polymorphic
D10S611	G08794	Bayes *et al.* (2000)	*Papio anubis*	Polymorphic
		Kanthaswamy *et al*. (2006)	*Macaca mulatta*	Polymorphic
		Liu *et al.* (2008)	*Rhinopithecus bieti*	*Not indicated*
D11S956	*	Grobler *et al*. (2006)	*Chlorocebus aethiops*	*Not indicated*
D14S306	G09055	Bayes *et al.* (2000)	*Papio anubis*	Polymorphic
D15S108	L15778	Newman *et al.* (2002)	*Chlorocebus aethiops*	Polymorphic
		Lau *et al.* (2004)	*Aotus azarai,*	Polymorphic
			Aotus lemurinus	Polymorphic
			Aotus nancymaae	Polymorphic
		Grobler *et al*. (2006)	*Chlorocebus aethiops*	*Not indicated*
D18S72	Z17153	Newman *et al.* (2002)	*Chlorocebus aethiops*	Polymorphic
		Andrade *et al*. (2004)	*Macaca mulatta*	Polymorphic

* Accession code not indicated in the GenBank database

Table 2.3: Primer sequences and repeat motive of the selected loci

Locus	Sequence	Repeat
D1S518	F: 5'-TGCAGATCTTGGGACTTCTC-3'	Tetra
	R: 5'-AAAAAGAGTGTGGGCAACTG-3'	
D2S1326	F: 5'-AGACAGTCAAGAATAACTGCCC-3'	Tetra
	R: 5'-CTGTGGCTCAAAAGCTGAAT-3'	
D3S1768	F: 5'-GGTTGCTGCCAAAGATTAGA-3'	Tetra
	R: 5'-CACTGTGATTTGCTGTTGGA-3'	
D4S243	F: 5'-TCAGTCTCTCTTTCTCCTTGCA-3'	Tetra
	R: 5'-TAGGAGCCTGTGGTCCTGTT-3'	
D5S1457	F: 5'-TAGGTTCTGGGCATGTCTGT-3'	Di
	R: 5'-TGCTTGGCACACTTCAGG-3'	
D7S2204	F: 5'-TCATGACAAAACAGAAATTAAGTG-3'	Tetra
	R: 5'-AGTAAATGGAATTGCTTGTTACC-3'	
D10S611	F: 5'-CATACAGGAAACTGTGTAGTGC-3'	Tetra
	R: 5'-CTGTATTTATGTGTGTGGATGG-3'	
D11S956	F: 5'-GATCAGTAATTAGCCAGACTCTAGG-3'	Tetra
	R: 5'-GGTTTTGGAGCTTAAGGAGG-3'	
D14S306	F: 5'-AAAGCTACATCCAAATTAGGTAGG-3'	Tetra
	R: 5'-TGACAAAGAAACTAAAATGTCCC-3'	
D15S108	F: 5'-AGGAGAGCTAGAGCTTCTAT-3'	Di
	R: 5'-GTTTCAACATGAGTTTCAGA-3'	
D18S72	F: 5'-GCTAGATGACCCAGTTCCC-3'	Di
	R: 5'-CAAGAGAGCCCTTTGGTTT-3'	

2.2.6 PCR-based amplification of fragments

PCR is a rapid technique to amplify a specific DNA segment between two regions of known sequence. This technique relies on thermal cycling and consists of denaturation, annealing and elongation steps. The primers along with a heat-stable DNA polymerase enable synthesis of complementary strands of DNA.

Eleven primer pairs, consisting of a fluorescently labeled forward primer and an unlabeled reverse primer, were used to amplify each locus from genomic DNA. The annealing temperatures (T_a) for the selected primers were optimized with a touch down protocol starting at 5˚C under the melting temperature (T_m) of the primer pair. The temperature was then adjusted until the required level of specificity was obtained. The higher the T_a, the more specific the reaction became. The temperature was increased to

enhance the specificity and reduce background peaks. In some cases where the peaks were too high, the T_a was decreased or the primer pair was diluted in order to lower the peaks.

A range of parameters were considered in order to optimize a reaction, including the T_a of each primer pair, the concentration of each primer pair and the concentration of magnesium chloride ($MgCl_2$). The first series of amplifications was performed with a primer concentration of 10 micromolar (µM). However, the peaks were too high. A dilution series was set up for each primer pair starting with a concentration of 10 µM (Figure 2.2).

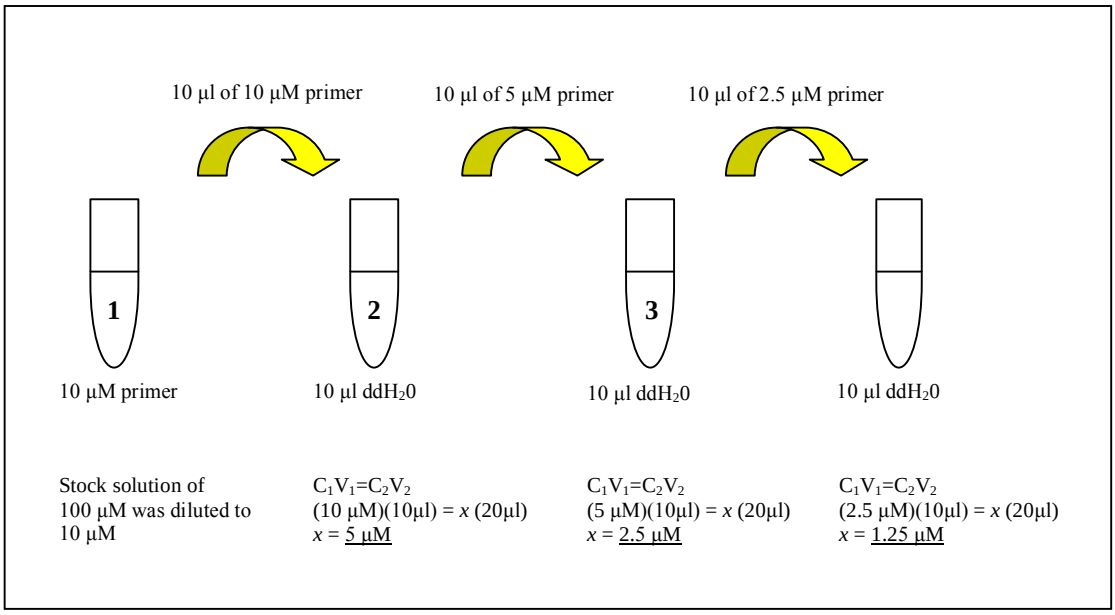

Figure 2.2: Primer dilution series

PCR was conducted using two standard DNA samples for all the primers. After PCR, the amplification products were run on the ABI PRISM®[2] 3130 Genetic Analyzer and the results were interpreted. The reactions with the 1.25 µM primer concentration provided the best results with peak heights between 2000-5000 relative fluorescent units (rfu) and no background peaks.

[2] ABI PRISM is a registered trademark of Applera Corporation, Foster City, California, USA.

Mg^{2+} acts as a co-factor for the *Taq* DNA polymerase. The higher the $MgCl_2$ concentration, the less specific the reaction became. In order to optimize the $MgCl_2$ concentration, a reaction range was set up with the concentrations: 1 millimolar (mM), 1.5 mM, 2 mM and 2.5 mM. After PCR, the amplification products were run on the ABI PRISM® 3130 Genetic Analyzer and the results were interpreted. The concentration that provided the best results was used for all the reactions to follow.

Promega GoTaq®[3] Flexi DNA Polymerase (Promega) was used for PCR together with 1x GoTaq® Flexi buffer. Each PCR reaction consisted of the following components: 50-150 nanograms (ng) DNA, 0.5 units (U) GoTaq® Flexi DNA Polymerase, 1.25x GoTaq® Flexi buffer, 0.25 mM deoxynucleotide triphosphate (dNTP's), 2-2.5 mM $MgCl_2$ and 0.083 µM of each primer. The reaction was filled up with double distilled water (ddH_2O) to a final volume of 15 microliter (µl).

PCR amplification was conducted using a Perkin Elmer 9700 thermal cycler with temperature cycles as follow: initial denaturation at 94˚C for 3 minutes (min); 10 cycles of 94˚C for 30 seconds (s), T_a+5˚C for 30 s, 72˚C for 30 s; 10 cycles of 94˚C of 30 s, T_a for 30 s, 72˚C for 30 s; 20 cycles of 94˚C for 30 s, T_a-5˚C for 30 s, 72˚C for 30 s, and final elongation at 72˚C for 20 min where after the temperature is decreased to 4˚C for an indefinite period.

For every PCR procedure human DNA was used as a positive control, ddH_2O as a negative control and two standards with known results was amplified as well.

2.2.7 Capillary electrophoresis

Capillary electrophoresis is used to separate DNA fragments and it is usually performed for analytical purposes. The fact that alleles differ in length makes it possible to separate and visualize it through high resolution capillary electrophoresis. Fluorescently labeled

[3] GoTaq is a registered trademark of Promega Corporation, Madison, WI, USA.

fragments were detected using the ABI PRISM® 3130 Genetic Analyzer and the alleles were sized using GeneMapper®[4] software (version 3).

An internal size standard was used to allow comparison of the samples. The analysis software uses the size standard to generate a standard curve and then determines the length of the labeled fragment by comparing it to the standard curve. GeneScan™[5] – LIZ®[6] (Applied Biosystems) was used as an internal size standard for all the primers except for D15S108 (labeled with HEX), in which case GeneScan™ 400HD (ROX™[5] Dye, Applied Biosystems) was used.

The matrix is used to analyze different fluorescently labeled samples in a single capillary. The Matrix Standard Set DS-33 (Dye set G5) is required when analyzing DNA fragments labeled with 6-FAM, VIC®[6], NED, PET and LIZ® on the ABI PRISM® 3130 Genetic Analyzer. The Matrix Standard Set DS-30 (Dye set D) is required when analyzing DNA fragments labeled with 6-FAM, HEX, NED and ROX™.

Microsatellite loci with non-overlapping size ranges and labeled with different dyes were amplified together as a multiplex. The T_a of all the primers did not differ significantly and ranged between 56-58˚C. As a result, the loci could be amplified together with ease. Multiplexing different dyes and fragment sizes together results in a higher output as it is less time consuming and less DNA is used per locus analyzed (Luikart *et al.*, 2008). It is also more cost effective. Seven primers were divided into three plexes and four were run separately (Table 2.4).

[4] GeneMapper is a registered trademark of Applera Corporation, Foster City, California, USA.
[5] GeneScan and ROX are trademarks of Applera Corporation, Foster City, California, USA.
[6] LIZ and VIC are registered trademarks of Applera Corporation, Foster City, California, USA.

Table 2.4: Primer plexes used for *P. ursinus* population

Plex	Forward Primer	Dye
A	D5S1457	6-FAM
	D10S611	NED
	D11S956	VIC®
B	D4S243	NED
	D18S72	VIC®
C	D1S518	NED
	D14S306	6-FAM
Run separately	D3S1768	6-FAM
	D2S1326	6-FAM
	D7S2204	6-FAM
	D15S108	HEX

2.2.8 Genotyping

In the figures below, an illustration of a representative homozygous and heterozygous profile is given for each locus.

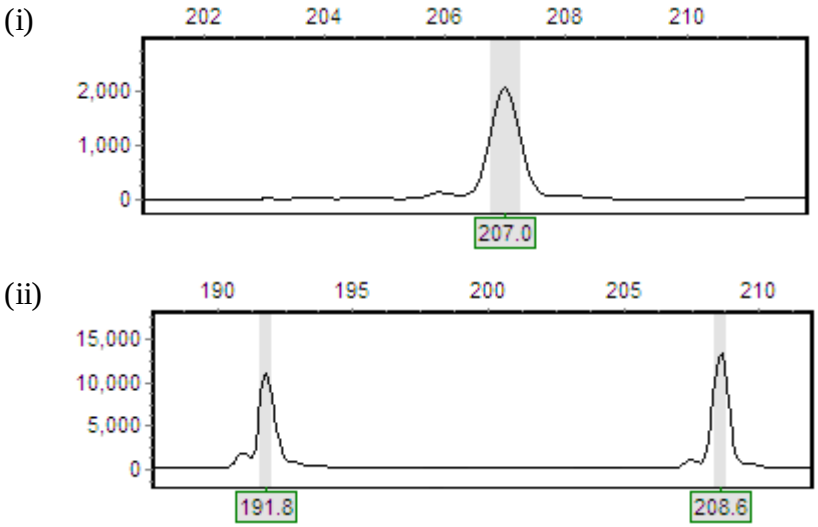

Figure 2.3: Homozygous (i) and heterozygous (ii) profiles for locus D1S518.

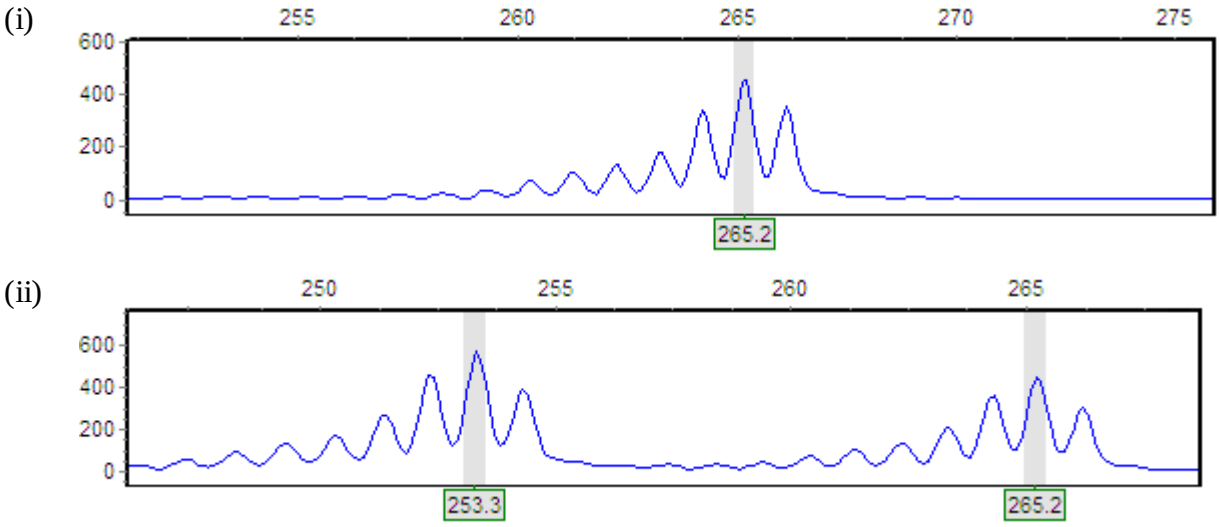

Figure 2.4: Homozygous (i) and heterozygous (ii) profiles for locus D2S1326. A cluster of three peaks was formed for each allele with the peak in the middle being higher and thus scored as the main peak.

In order to achieve constant and accurate results, the peak in the middle of the cluster of the three highest peaks was used to score the alleles.

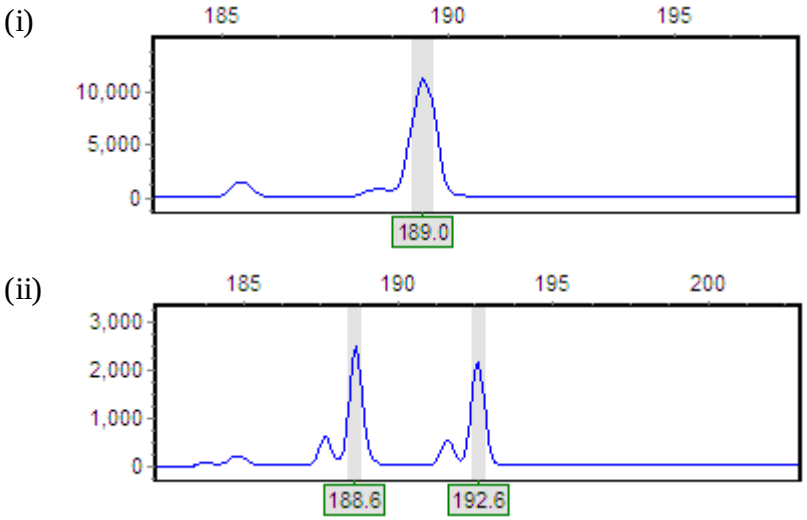

Figure 2.5: Homozygous (i) and heterozygous (ii) profiles for locus D3S1768.

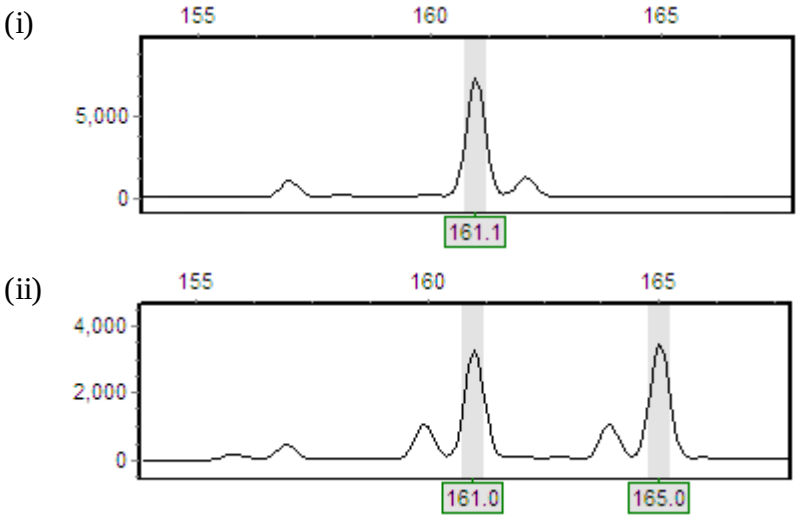

Figure 2.6: Homozygous (i) and heterozygous (ii) profiles for locus D4S243.

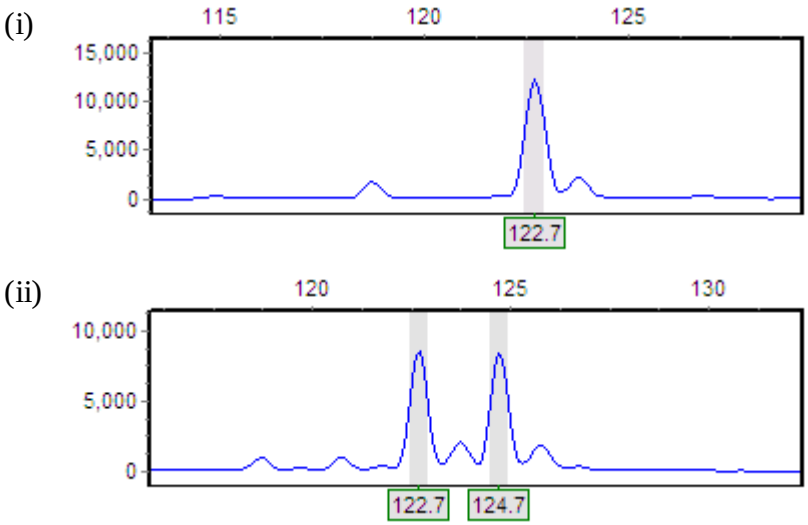

Figure 2.7: Homozygous (i) and heterozygous (ii) profiles for locus D5S1457.

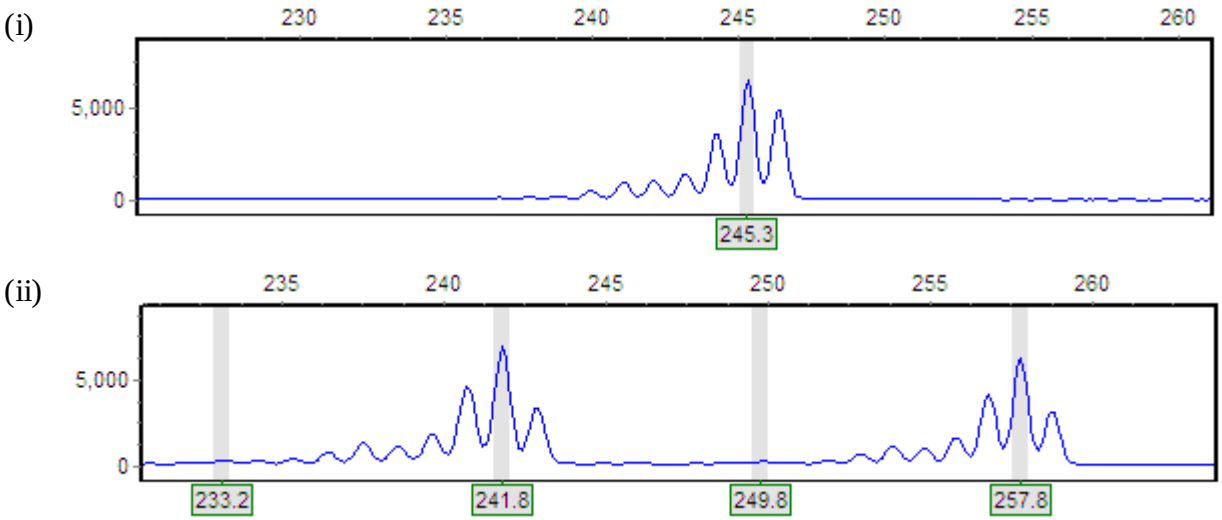

Figure 2.8: Homozygous (i) and heterozygous (ii) profiles for locus D7S2204. A cluster of three peaks was formed for each allele with the peak in the middle being higher and thus scored as the main peak.

A cluster of three peaks were observed for locus D7S2204. The second (highest) peak in the cluster was used to score the alleles and ensure constant and accurate results.

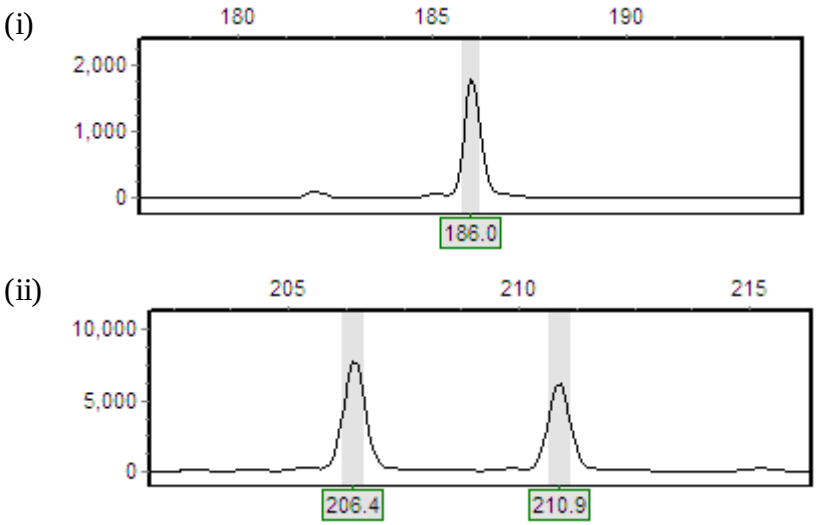

Figure 2.9: Homozygous (i) and heterozygous (ii) profiles for locus D10S611.

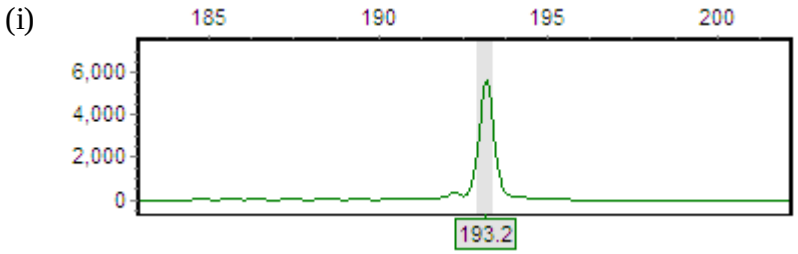

Figure 2.10: Homozygous profile (i) for locus D11S956. The Suikerbosrand population as well as the outgroup was homozygous for this locus.

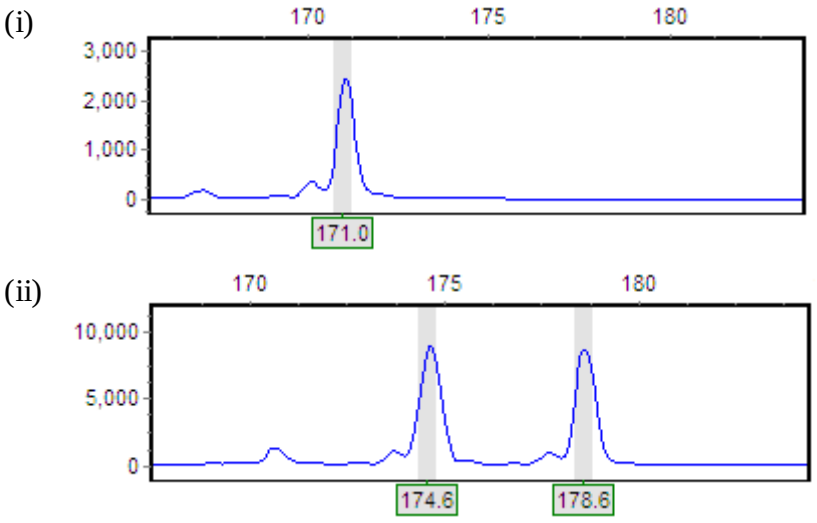

Figure 2.11: Homozygous (i) and heterozygous (ii) profiles for locus D14S306.

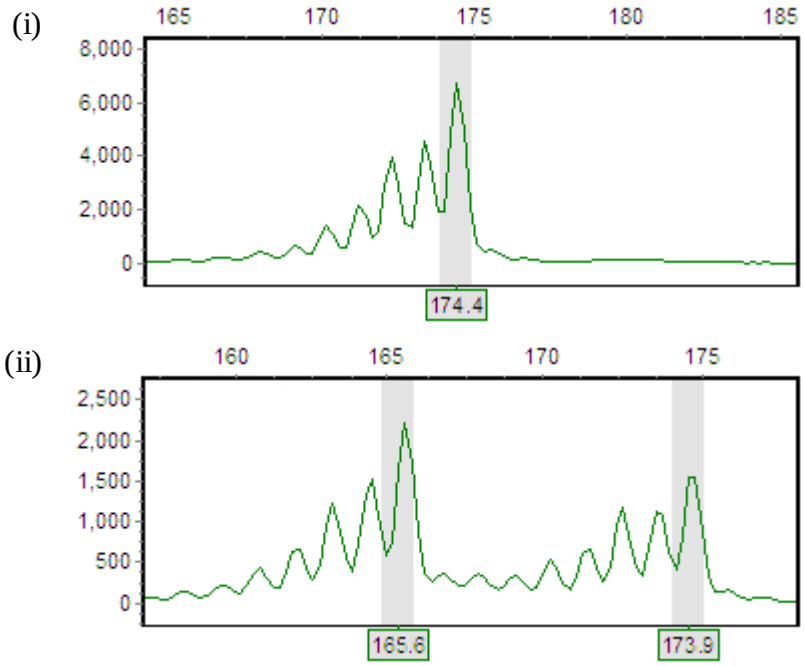

Figure 2.12: Homozygous (i) and heterozygous (ii) profiles for locus D15S108. A cluster of five peaks, gradually increasing in height, was observed. The highest peak was used to score the size of the allele.

Locus D15S108 produced a cluster of five peaks that gradually increased in height. The last (highest) peak was used to ensure that allelic scoring is constant and accurate.

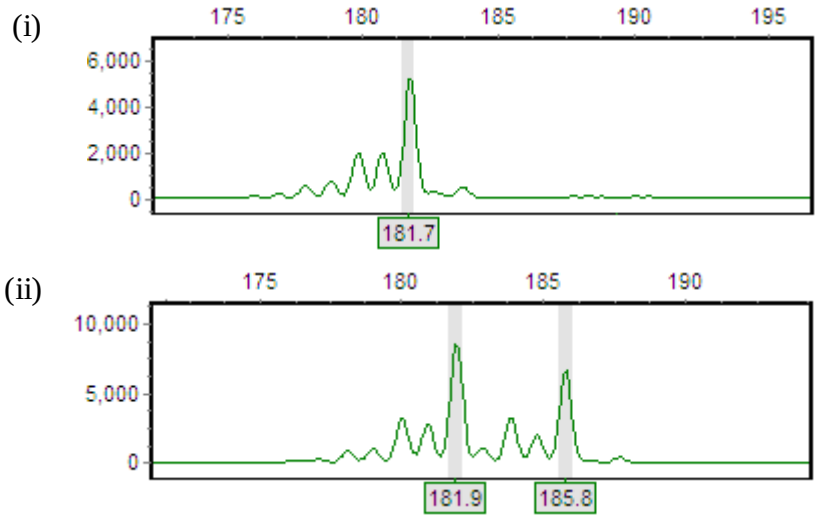

Figure 2.13: Homozygous (i) and heterozygous (ii) profiles for locus D18S72. The main peaks at this locus followed after two smaller peaks. In the case of a heterozygote (ii), the second main peak was lower than the first one.

Locus D18S72 produced a cluster of three peaks, the first two peaks being more or less the same height and the third peak being the highest. The third peak was used to ensure accurate and constant allelic scoring.

Accurate scoring of the alleles at loci that produced cluster peaks was also ensured by using tetranucleotide markers. Since alleles from a heterozygous individual should be four base pairs apart, the use of tetranucleotides lessens the impact of non-informative peaks between true peaks.

2.2.9 Statistical Analysis

A wide range of software packages have been designed over the past few decades to assist researchers in population genetic studies. The Excel Microsatellite Toolkit (Park, 2001) is an integrated function for Microsoft Excel. It contains tools for population genetic studies that were conducted using microsatellite markers. Some of the Toolkit functions include data checking, data formatting, calculation of basic diversity values and sample matching. Using the data checking tool, invalid alleles, incompletely-typed samples and invalid sample or population names can be detected. Data can be formatted and input files created for population genetics software, which include Arlequin, Microsat, Genepop, FStat and Dispan. The observed and expected heterozygosity, polymorphism information content and the mean number of alleles per locus can be calculated using the Microsatellite Toolkit.

Microsatellite data was analyzed using Arlequin (Excoffier *et al*., 2005); population genetics software that provides methods such as analysis of population subdivision under the Analysis of Molecular Variance (AMOVA) framework (Excoffier *et al*., 1992) and the computation of F-statistics to analyze differentiation from molecular data. Genetic differentiation within the population was quantified using AMOVA; a program developed by Laurent Excoffier.

The Hardy-Weinberg equilibrium, genotypic linkage disequilibrium, population differentiation, Nm estimates, F_{ST} and other correlations, and basic information such as allele frequencies and observed and expected genotype proportions were determined using GENEPOP (Raymond and Rousset, 1995). Measures used for the calculation of genetic diversity included unbiased heterozygosity (Hz), observed heterozygosity (Ho) and the average number of alleles per locus.

The Hardy-Weinberg equilibrium is the equilibrium reached by allele and genotype frequencies under random mating, no genetic drift, no mutation, no selection and no migration.

Nm is the level of migration, expressed as the number of migrants per generation.

F_{ST} is defined by Wright (1965) as the correlation between two alleles chosen at random within subpopulations relative to alleles that were randomly sampled from the total population. F_{ST} is used to measure the degree of differentiation between populations. The higher the F_{ST} value, the more genetically distinct populations are, and this can provide valuable insight on processes such as genetic drift.

R_{ST} (Slatkin, 1995) is a measure of genetic differentiation similar to F_{ST}, but is based on the assumption of a step-wise mutation model in the microsatellite areas studied. R_{ST} Calc (Goodman, 1997) was used to calculate the R_{ST} values.

The Bayesian clustering method implemented in the program STRUCTURE uses the Hardy-Weinberg equilibrium and linkage disequilibrium within subpopulations to determine the number of populations (clusters or K). This program was developed by Pritchard *et al*. (2000) to use multi-locus genotype data and study population structure. STRUCTURE presents an estimate of the number of genetic clusters in a population. The number of sampled individuals is divided into a number of clusters (K) based on multi-locus genotypic data (no locality information). The program can be used to determine the

true number of genetic populations, understand distinct populations, assign individuals to a population, study hybrids and identify migrants.

2.3 Results and Discussion

Sufficient amounts of DNA for reliable genotyping results were extracted from all the samples. In cases where the DNA concentration was insufficient, the sample was re-extracted.

A total of 145 samples were profiled successfully with 11 microsatellite markers. The number of alleles observed for each locus is listed in Table 2.5. All the microsatellite markers were polymorphic except for D11S956 which was monomorphic in the Suikerbosrand troops as well as the outgroup. (Note that this locus was not tested for the presence of null alleles.)

Table 2.5: Number of alleles observed for each locus and the allele range.

Locus	Number of alleles	Allele size range
D1S518	7	191-219
D2S1326	8	237-269
D3S1768	4	185-201
D4S243	5	157-173
D5S1457	11	107-137
D7S2204	6	230-254
D10S611	10	184-230
D11S956	1	193
D14S306	8	151-183
D15S108	3	166-178
D18S72	3	182-190

All the samples amplified at all the loci, with the homozygous alleles amplified and genotyped two to three times in order to ascertain homozygous profiles. In the few cases

where split peaks were observed, the time for the final elongation step was increased. The genotyping results are given in Appendix B.

2.3.1 Statistical analysis

The first objective of this study was to construct a genetic database with individual profiles for all the sampled animals. This was achieved by typing each individual at 11 different microsatellite loci. The results obtained from the microsatellite loci (see Appendix B) were used for further statistical analysis. A second objective of this study was to determine genetic relationships in the overall Suikerbosrand population.

2.3.1.1 Linkage disequilibrium

Linkage disequilibrium is defined as the non-random association of alleles among loci. Non-random association among loci can be a result of chance events, population bottlenecks, recent mixing of different populations or selection.

The sequential Bonferroni test was applied to correct errors (P=0.001). Of the 11 loci, three pairs were linked with each other: D5S1457 and D1S518; D10S611 and D14S306; D15S108 and D2S1326 (Table 2.6). Loci D10S611 and D14S306 have been used together previously by Buchan *et al*. (2005) in a study on savannah baboons (*Papio cynocephalus*). These loci were also included in this study as the *Papio cynocephalus* species has the same number of chromosomes as *Papio ursinus*, i.e. 42 chromosomes (De Grouchy *et al*., 1978).

Table 2.6: Linkage disequilibrium results for the 11 microsatellite loci. The loci are: D5S1457 (0), D10S611 (1), D11S956 (2), D4S243 (3), D18S72 (4), D1S518 (5), D14S306 (6), D3S1768 (7), D15S108 (8), D2S1326 (9) and D7S2204 (10).

	0	1	2	3	4	5	6	7	8	9	10
0	*	-	-	-	-	+	-	-	-	-	-
1	-	*	-	-	-	-	+	-	-	-	-
2	-	-	*	-	-	-	-	-	-	-	-
3	-	-	-	*	-	-	-	-	-	-	-
4	-	-	-	-	*	-	-	-	-	-	-
5	+	-	-	-	-	*	-	-	-	-	-
6	-	+	-	-	-	-	*	-	-	-	-
7	-	-	-	-	-	-	-	*	-	-	-
8	-	-	-	-	-	-	-	-	*	+	-
9	-	-	-	-	-	-	-	-	+	*	-
10	-	-	-	-	-	-	-	-	-	-	*

2.3.1.2 Genetic differentiation

The genetic differentiation among troops and among populations was determined using AMOVA as implemented in Arlequin software. Results shown in Table 2.7 indicate that the difference between the Suikerbosrand troops contributed almost 6% to the overall diversity, with the variation within individual troops contributing 83%. The difference between the Suikerbosrand population and the outgroup was 11%.

Table 2.7: AMOVA results for Suikerbosrand population and the outgroup

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	1	13.347	0.416	11.120
Among troops within populations	13	96.110	0.210	5.610
Within troops	283	881.493	3.115	83.270
Total	297	990.950	3.741	

Pair-wise F_{ST} and associated *P*-values were calculated among the troops as an additional measure of genetic differentiation and connectivity. Among population R_{ST} values was calculated as an addition to F_{ST}. Table 2.8 gives the F_{ST} values. A sequential Bonferroni correction was applied to compensate for the many pair-wise comparisons done and the *P*-value was adjusted to 0.0005. There is no significant drift or differentiation ($P>0.0005$) among the fourteen troops at Suikerbosrand, or between the Suikerbosrand troops and the outgroup.

Table 2.8: Estimates of population differentiation between the different troops at Suikerbosrand and the outgroup with the F_{ST}-values above the diagonal. The fourteen troops of the Suikerbosrand baboon population are: Bezuidenhoutshoek (1), Boschhoek (2), Diepkloof (3), Feeshuis (4), Groot Plato's (5), Heuningkrans (6), Kareekloof (7), Schikfontein (8), Schoongezicht (9), Steenbokhut (10), Toringkop (11), Valsfontein (12), Wetter (13), Wolwekloof (14); Outgroup (15)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	**	0.076 P=0.001	0.055 P=0.001	0.015 P=0.114	0.022 P=0.086	0.055 P=0.001	0.080 P=0.001	0.135 P=0.003	0.114 P=0.001	0.041 P=0.009	0.029 P=0.061	0.009 P=0.293	0.051 P=0.010	0.045 P=0.009	0.212 P=0.001
2		**	0.067 P=0.001	0.040 P=0.001	0.0775 P=0.004	0.069 P=0.001	0.119 P=0.001	0.158 P=0.001	0.143 P=0.001	0.083 P=0.001	0.062 P=0.001	0.071 P=0.002	0.129 P=0.001	0.096 P=0.001	0.164 P=0.001
3			**	0.029 P=0.014	0.020 P=0.091	0.030 P=0.004	0.030 P=0.014	0.093 P=0.002	0.063 P=0.001	0.077 P=0.001	0.027 P=0.041	0.019 P=0.142	0.055 P=0.009	0.028 P=0.036	0.131 P=0.001
4				**	0.024 P=0.089	0.031 P=0.003	0.061 P=0.001	0.103 P=0.001	0.076 P=0.001	0.001 P=0.422	0.033 P=0.030	0.013 P=0.244	0.069 P=0.001	0.034 P=0.013	0.162 P=0.001
5					**	0.054 P=0.009	0.051 P=0.009	0.127 P=0.017	0.107 P=0.001	0.052 P=0.020	0.023 P=0.106	0.052 P=0.045	0.049 P=0.021	0.016 P=0.252	0.191 P=0.007
6						**	0.054 P=0.001	0.069 P=0.010	0.058 P=0.001	0.060 P=0.001	0.053 P=0.002	0.044 P=0.011	0.104 P=0.001	0.022 P=0.078	0.138 P=0.001
7							**	0.119 P=0.006	0.032 P=0.013	0.131 P=0.001	0.054 P=0.006	0.080 P=0.003	0.020 P=0.106	0.021 P=0.061	0.155 P=0.001
8								**	0.137 P=0.001	0.127 P=0.002	0.066 P=0.058	0.058 P=0.097	0.137 P=0.013	0.071 P=0.048	0.163 P=0.007
9									**	0.108 P=0.001	0.122 P=0.001	0.099 P=0.001	0.113 P=0.001	0.076 P=0.001	0.175 P=0.001
10										**	0.070 P=0.005	0.040 P=0.066	0.141 P=0.001	0.070 P=0.001	0.194 P=0.001
11											**	0.034 P=0.092	0.071 P=0.010	0.027 P=0.146	0.138 P=0.003
12												**	0.097 P=0.004	0.051 P=0.045	0.159 P=0.001
13													**	0.045 P=0.073	0.224 P=0.003
14														**	0.130 P=0.001
15															**

2.3.1.3 Gene flow

The R_{ST} value with its *P*-value is given below the diagonal in Table 2.9. The Nm value is given above the diagonal. A negative R_{ST} value and a (∞) Nm-value is an indication of high levels of gene flow between troops. The sequential Bonferroni test was applied to correct errors (*P*-value was adjusted to 0.0005). The troops of Suikerbosrand display high levels of gene flow among troops. The highest levels of gene flow are between the troops living in the central area of the reserve (Figure 2.14). The reason might be the fact that their home ranges will overlap and encounters with other troops are common. The Bezuidenhoutshoek troop displays high levels of gene flow (∞) with four troops: Groot Plato's, Kareekloof, Wolwekloof and Wetter. The Bezuidenhoutshoek troop is located in the centre of the reserve and their home range overlaps with a number of troops. The lowest level of gene flow is observed between the Schikfontein troop and the Toringkop troop. This could be a result of the fact that Schikfontein is located at the border of the reserve and the troop's home range does not overlap the home range of the Toringkop troop. There is no genetic differentiation between the troops with negative R_{ST} values indicated in blue in Table 2.9. The highest level of genetic differentiation is observed between the Schikfontein and Toringkop troops.

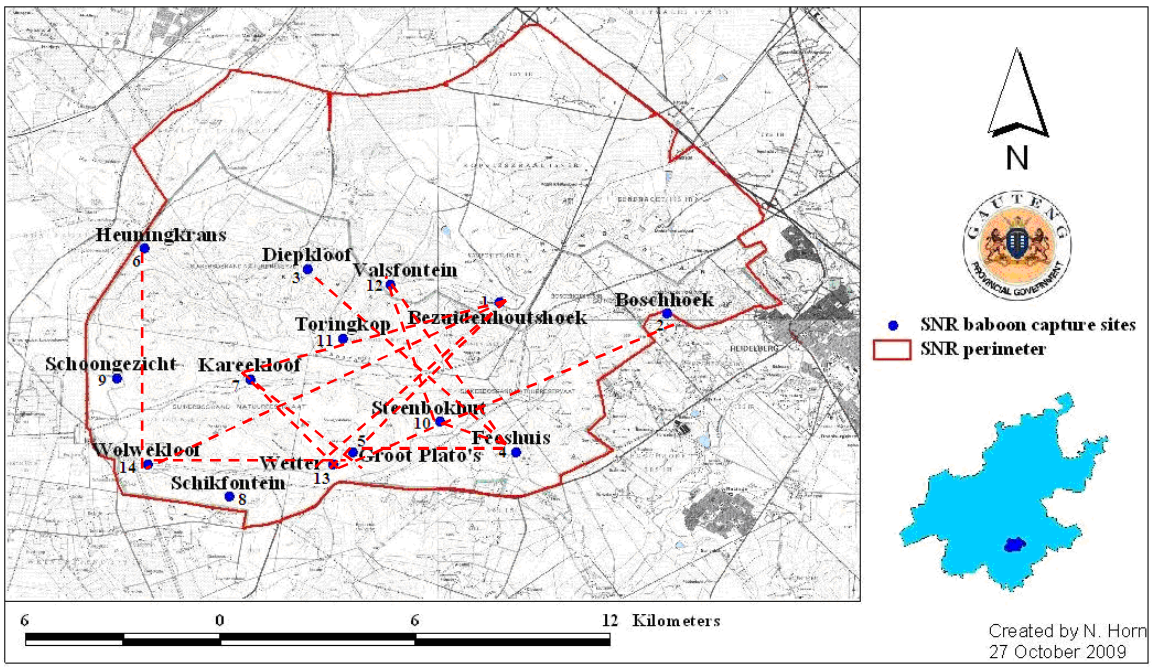

Figure 2.14: Troops with the highest levels of gene flow (Nm = ∞) (interconnected by red dotted lines)

Table 2.9: Gene flow among troops. R_{ST} values are given below the diagonal and the Nm value is given above the diagonal. The fourteen troops of the Suikerbosrand baboon population are: Bezuidenhoutshoek (1), Boschhoek (2), Diepkloof (3), Feeshuis (4), Groot Plato's (5), Heuningkrans (6), Kareekloof (7), Schikfontein (8), Schoongezicht (9), Steenbokhut (10), Toringkop (11), Valsfontein (12), Wetter (13), Wolwekloof (14); Outgroup (15)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	**	87.448	3.510	62.878	∞	14.984	∞	3.655	15.809	391.782	2.700	53.118	∞	∞	3.699
2	0.003 P=0.140	**	2.902	5.912	25.760	3.228	12.932	1.302	3.113	3.638	5.330	2.848	∞	5.865	7.998
3	0.06 P=0.050	0.079 P=0.001	**	∞	10.961	4.171	2.918	1.613	1.967	20.076	106.299	23.794	3.461	3.286	2.450
4	0.004 P=0.200	0.041 P=0.060	-0.001 P=0.450	**	∞	8.564	6.946	1.818	5.729	∞	12.365	∞	17.872	8.440	2.542
5	-0.015 P=0.400	0.010 P=0.220	0.022 P=0.260	-0.011 P=0.510	**	77.232	∞	2.243	10.298	86.841	3.151	6.784	∞	∞	4.904
6	0.016 P=0.150	0.072 P=0.020	0.057 P=0.020	0.028 P=0.110	0.003 P=0.410	**	8.134	14.686	11.316	10.531	2.544	5.127	4.216	∞	2.512
7	-0.021 P=0.680	0.019 P=0.130	0.079 0.001	0.035 P=0.050	-0.004 P=0.250	0.030 P=0.030	**	3.942	12.289	6.053	1.967	8.386	∞	7.634	4.320
8	0.064 P=0.060	0.161 P=0.001	0.134 P=0.050	0.121 P=0.010	0.100 P=0.060	0.017 P=0.240	0.060 P=0.070	**	6.010	3.003	0.934	3.045	1.325	3.623	1.152
9	0.016 P=0.110	0.074 P=0.001	0.113 P=0.001	0.042 P=0.060	0.024 P=0.170	0.022 P=0.040	0.020 P=0.040	0.040 P=0.070	**	4.544	1.578	2.630	4.529	7.587	1.776
10	0.001 P=0.320	0.064 P=0.010	0.012 P=0.230	-0.019 P=0.730	0.003 P=0.350	0.023 P=0.170	0.040 P=0.040	0.077 P=0.080	0.052 P=0.020	**	4.396	∞	5.501	5.858	1.798
11	0.085 P=0.060	0.045 P=0.070	0.002 P=0.480	0.020 P=0.160	0.074 P=0.100	0.090 P=0.020	0.113 P=0.001	0.211 P=0.030	0.137 P=0.001	0.054 P=0.080	**	3.075	2.430	2.035	2.186
12	0.005 P=0.260	0.081 P=0.010	0.010 P=0.440	-0.008 P=0.520	0.036 P=0.200	0.047 P=0.060	0.029 P=0.100	0.076 P=0.100	0.087 P=0.030	-0.032 P=0.730	0.075 P=0.050	**	3.966	3.403	2.048
13	-0.029 P=0.630	-0.025 P=0.660	0.067 P=0.070	0.014 P=0.280	-0.046 P=0.810	0.056 P=0.020	-0.013 P=0.430	0.159 P=0.060	0.052 P=0.050	0.044 P=0.170	0.093 P=0.060	0.059 P=0.140	**	115.977	9.756
14	-0.001 P=0.320	0.041 P=0.060	0.071 P=0.060	0.029 P=0.150	-0.017 P=0.640	-0.007 P=0.470	0.031 P=0.130	0.065 P=0.150	0.032 P=0.100	0.041 P=0.100	0.109 P=0.060	0.068 P=0.080	0.002 P=0.380	**	4.125
15	0.063 P=0.030	0.030 P=0.140	0.093 P=0.20	0.090 P=0.030	0.049 P=0.160	0.091 P=0.010	0.055 P=0.020	0.178 P=0.001	0.123 P=0.001	0.122 P=0.001	0.103 P=0.080	0.109 P=0.080	0.025 P=0.130	0.057 P=0.090	**

2.3.1.4 Genetic diversity

The allele frequencies for the Suikerbosrand population are given in Table 2.10. A total of 66 alleles were detected in 11 microsatellite loci. The alleles indicated in red was only observed in the results obtained for the outgroup. Locus D11S956 proved to be monomorphic in chacma baboons and was not very informative.

Unique alleles were observed for Heuningkrans at locus D5S1457 (allele 119), and Schikfontein at D15S108 (allele 178).

Table 2.10: Allele frequencies for the Suikerbosrand troops

		Troops													
Locus	Allele	Bezuidenhoutshoek	Boschhoek	Diepkloof	Feeshuis	Groot Plato's	Heuningkrans	Kareekloof	Schikfontein	Schoongezicht	Steenbokhut	Toringkop	Valsfontein	Wetter	Wolwekloof
D5S1457	107														
	111	0.182	0.133			0.143	0.039	0.179		0.208	0.091		0.083	0.083	0.083
	115		0.100			0.071			0.125						0.043
	119						0.077								
	121					0.071	0.039	0.143							0.291
	123	0.455	0.467	0.231	0.464	0.214	0.500	0.321	0.375	0.542	0.455	0.333	0.250	0.417	0.167
	125	0.091	0.133	0.154	0.179	0.714	0.115	0.214	0.375	0.042	0.046	0.083	0.083	0.500	0.208
	127	0.227	0.033	0.423	0.179	0.214	0.077	0.036	0.125	0.042	0.182	0.333	0.500		0.083
	129														
	133				0.179	0.143	0.039	0.036			0.182	0.083			
	137	0.046	0.133	0.192		0.071	0.115	0.071		0.167	0.046	0.167	0.083		0.125
D10S611	184														
	186	0.046	0.133	0.154	0.214	0.143	0.115	0.143		0.042	0.273				
	190	0.091	0.200	0.039									0.333		
	206	0.455	0.033	0.192	0.214	0.357	0.231	0.464	0.375	0.250	0.182	0.583	0.417	0.500	0.375
	210	0.046	0.033	0.269	0.071	0.071	0.077		0.125	0.083	0.091	0.083		0.083	0.042
	214			0.115	0.071	0.071	0.154	0.179	0.500	0.167	0.046	0.083	0.083	0.333	0.208
	218		0.033				0.039	0.071							
	222	0.318	0.300	0.231	0.286	0.286	0.346	0.143		0.458	0.364	0.167	0.083	0.083	0.375
	226	0.046	0.200		0.107	0.071	0.039				0.046	0.083	0.083		
	230		0.067		0.036										
D11S956	193	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

D4S243	157	0.046	0.267	0.231	0.179	0.071	0.231	0.250	0.125	0.417	0.136	0.167	0.250		0.125
	161	0.955	0.533	0.500	0.786	0.786	0.462	0.607	0.375	0.333	0.773	0.667	0.583	1.000	0.667
	165		0.200	0.269	0.036	0.143	0.308	0.143	0.500	0.250	0.091	0.167	0.167		0.208
	169														
	173														
D18S72	182	0.091	0.167	0.192	0.107		0.385	0.214	0.250	0.167	0.046	0.333	0.083		0.042
	186	0.636	0.600	0.346	0.500	0.571	0.346	0.357	0.500	0.500	0.864	0.583	0.500	0.583	0.583
	190	0.273	0.233	0.462	0.393	0.429	0.269	0.429	0.250	0.333	0.091	0.083	0.417	0.417	0.375
D1S518	191	0.182	0.133	0.154	0.357		0.154	0.143	0.250	0.417	0.546		0.500		
	199	0.455	0.100	0.077	0.179	0.214	0.308		0.500		0.182	0.417	0.167	0.167	0.333
	203	0.364	0.267	0.615	0.286	0.786	0.269	0.821	0.250	0.542	0.182	0.583	0.250	0.750	0.542
	207		0.500	0.154	0.179		0.269	0.036		0.042	0.091		0.083	0.083	0.125
	211														
	215														
	219														
D14S306	151				0.036					0.042					
	159	0.182	0.067	0.192	0.036	0.357	0.231	0.179		0.250	0.136	0.083		0.083	0.083
	163	0.046	0.300	0.192	0.214	0.286	0.115	0.179	0.625	0.167	0.273	0.250	0.083	0.333	0.292
	167														
	171	0.364	0.400	0.308	0.250	0.071	0.269	0.321			0.046	0.250	0.333	0.333	0.250
	175	0.409	0.233	0.308	0.464	0.214	0.385	0.321	0.375	0.542	0.546	0.417	0.500	0.250	0.375
	179					0.071							0.083		
	183														
D3S1768	185	0.046	0.067	0.269	0.107		0.115	0.036		0.042	0.227	0.083			0.125
	189	0.227	0.233	0.231	0.214	0.214	0.115	0.143	0.875	0.042	0.227	0.500	0.500	0.250	0.250
	197	0.455	0.167	0.308	0.250	0.214	0.269	0.500		0.667	0.136		0.333	0.583	0.292
	201	0.273	0.533	0.192	0.429	0.571	0.500	0.321	0.125	0.250	0.409	0.417	0.167	0.167	0.333
D15S108	166	0.727	0.833	0.769	0.857	0.786	0.500	0.750	0.500	0.875	0.773	0.833	0.667	0.833	0.583
	174	0.273	0.167	0.231	0.143	0.214	0.500	0.250	0.250	0.125	0.227	0.167	0.333	0.167	0.417
	178								0.250						
D2S1326	237	0.182	0.033	0.192	0.143	0.143	0.385	0.286	0.625	0.458	0.136		0.250	0.167	0.167
	245														
	249	0.136		0.115	0.071	0.214	0.039	0.036		0.041	0.182	0.167	0.083	0083	0.083
	253	0.046	0.100	0.039	0.214	0.071	0.077	0.214	0.125	0.208	0.091	0.083			0.292
	257	0.091	0.067	0.039	0.143		0.115	0.071			0.182	0.167	0.167		0.125
	261	0.546	0.800	0.615	0.429	0.571	0.385	0.393	0.250	0.292	0.409	0.583	0.500	0.750	0.333
	265														
	269														
D7S2204	230														
	238	0.591	0.467	0.423	0.571	0.714	0.500	0.071	0.375	0.167	0.727	0.250	0.667	0.250	0.417
	242	0.046	0.033	0.385	0.250	0.214	0.423	0.536	0.250	0.542	0.227	0.333	0.083	0.417	0.542
	246	0.046		0.039				0.036							
	250	0.091		0.039			0.039	0.214	0.250	0.250			0.083	0.333	
	254	0.227	0.500	0.114	0.179	0.071	0.039	0.143	0.125	0.042	0.045	0.417	0.167		0.042

The average observed heterozygosity in the Suikerbosrand population is 0.583 and does not differ significantly from the observed heterozygosity of 0.691 of the outgroup. Similarly, the average number of alleles per locus is closely comparable between the Suikerbosrand population (3.51) and the outgroup (4.09). There is also no appreciable difference in heterozygosity or in the number of alleles between the different troops. Within Suikerbosrand, the Heuningkrans troop has the highest level of heterozygosity (0.678) and the highest number of alleles (4.090).

Table 2.11: Unbiased (Hz) and observed heterozygosity (Ho), the standard deviations (SD) and the number of alleles per locus for the Suikerbosrand population and the outgroup. The number of sampled individuals is indicated in brackets next to each troop.

Troop	Unbiased Hz	Unbiased Hz SD	Observed Hz	Observed Hz SD	No. of alleles	No. of alleles SD
Bezuidenhoutshoek (11)	0.529	0.077	0.570	0.045	3.640	1.570
Boschhoek (15)	0.546	0.073	0.576	0.039	3.820	1.890
Diepkloof (13)	0.604	0.071	0.622	0.040	3.730	1.420
Feeshuis (14)	0.572	0.078	0.578	0.040	3.730	1.620
Groot Plato's (7)	0.537	0.079	0.558	0.057	3.550	2.070
Heuningkrans (13)	0.628	0.067	0.678	0.039	4.090	2.020
Kareekloof (14)	0.573	0.074	0.604	0.039	3.820	1.660
Schikfontein (4)	0.590	0.075	0.568	0.075	2.820	0.870
Schoongezicht (12)	0.542	0.068	0.538	0.043	3.450	1.210
Steenbokhut (11)	0.527	0.077	0.512	0.045	3.730	1.560
Toringkop (6)	0.562	0.069	0.667	0.058	3.180	1.250
Valsfontein (6)	0.595	0.064	0.530	0.061	3.450	1.210
Wetter (6)	0.466	0.081	0.515	0.062	2.640	1.030
Wolwekloof (12)	0.596	0.070	0.538	0.043	3.550	1.570
Average	0.565	0.074	0.583	0.050	3.550	1.530
Outgroup (5)	0.600	0.091	0.691	0.062	4.090	2.070

The Hardy-Weinberg equilibrium test showed significant departure (P<0.05) from equilibrium at loci D1S518 (Feeshuis, Heuningkrans, Schikfontein, Steenbokhut), D2S1326 (Wolwekloof), D3S1768 (Steenbokhut), D4S243 (Wolwekloof), D5S1457

(Schoongezicht, Wolwekloof), D14S306 (Valsfontein) and D15S108 (Schikfontein). The other comparisons were not significant (See Table 2.12). Since no populations showed a disproportionate number of deviations from expected Hardy-Weinberg equilibrium, it can be concluded that processes such as selection and non-random mating play a limited role in the Suikerbosrand population.

Table 2.12: All the troops in Hardy-Weinberg equilibrium at the given locus are indicated with an asterix (*). The *P*-value is given for troops that are not in equilibrium. The fourteen troops of the Suikerbosrand baboon population are: Bezuidenhoutshoek (1), Boschhoek (2), Diepkloof (3), Feeshuis (4), Groot Plato's (5), Heuningkrans (6), Kareekloof (7), Schikfontein (8), Schoongezicht (9), Steenbokhut (10), Toringkop (11), Valsfontein (12), Wetter (13), Wolwekloof (14)

Locus	Troop													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D1S518	*	*	*	0.009	*	0.008	*	0.031	*	0.018	*	*	*	*
D2S1326	*	*	*	*	*	*	*	*	*	*	*	*	*	0.005
D3S1768	*	*	*	*	*	*	*	*	*	0.038	*	*	*	*
D4S243	*	*	*	*	*	*	*	*	*	*	*	*	*	0.010
D5S1457	*	*	*	*	*	*	*	*	0.013	*	*	*	*	0.031
D7S2204	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D10S611	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D11S956	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D14S306	*	*	*	*	*	*	*	*	*	*	*	0.022	*	*
D15S108	*	*	*	*	*	*	*	0.084	*	*	*	*	*	*
D18S72	*	*	*	*	*	*	*	*	*	*	*	*	*	*

2.3.1.5 Population structure

Before any conclusions can be made about the genetic structure of populations, it is important to determine the true number of populations. A population is usually defined based upon geographical proximity of individuals. However, populations can also display genetic sub-structure as a result of unidentified barriers to gene flow (Evanno *et al*., 2005).

Figure 2.15 illustrates the probability (-LnPr) of K=1-15, averaged over 5 runs for each value of K. The most likely K value for the baboons studied is K=1, suggesting that all baboons genotyped form part of a single genetically interchangeable unit.

Table 2.13: STRUCTURE results for five independent runs. (K=1)

	1	**2**	**3**	**4**	**5**	$\bar{x}$ **+ *SD***
K1	-3950.5	-3948.4	-3950.2	-3950.0	-3950.1	-3949.84
K2	-3879.2	-3894.2	-3903.2	-3904.7	-3906.0	-3897.46
K3	-3883.5	-3851.3	-3863.4	-3872.3	-3873.7	-3868.84
K4	-3929.6	-3914.2	-3904.6	-3940.9	-3923.7	-3922.6
K5	-3891.7	-3898.9	-3867.8	-3853.8	-3877.2	-3877.88
K6	-3908.5	-4083.9	-3903.9	-3893.9	-3936.6	-3945.36
K7	-4129.4	-4148.2	-4011.2	-3876.1	-4276.9	-4088.36
K8	-4316.0	-4472.1	-4307.1	-4426.3	-4220.5	-4348.4
K9	-4339.1	-4381.7	-4473.0	-4196.5	-4342.5	-4346.56
K10	-4589.0	-4447.3	-4513.1	-4538.4	-4208.4	-4459.24
K11	-4598.7	-4545.7	-4824.9	-4314.5	-4717.6	-4600.28
K12	-4444.5	-5225.9	-4188.1	-4496.7	-4842.8	-4639.6
K13	-4354.7	-4545.4	-4898.4	-4955.6	-3949.1	-4540.64
K14	-4860.5	-4846.7	-3963.1	-4972.3	-4626.3	-4653.78
K15	-3955.2	-5276.8	-4454.4	-3942.5	-4561.2	-4438.02

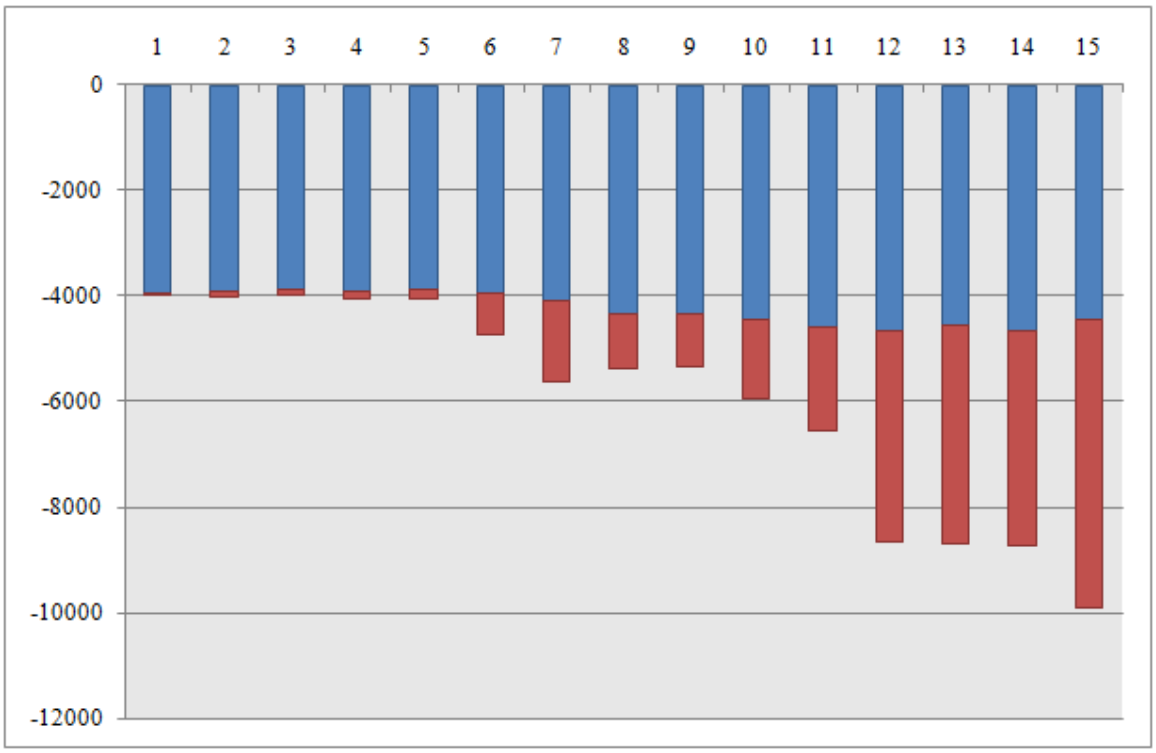

Figure 2.15: Probability (-LnPr) of K=1-15, averaged over 5 runs, with standard deviation over 5 runs for each value of K.

In conclusion, the baboon troops in Suikerbosrand are genetically similar as indicated by the R_{ST}, F_{ST} and STRUCTURE results above and high levels of gene flow are displayed. There are no barriers to gene flow between any of the troops, although the levels of gene flow differ and are generally higher in the centre of the reserve. All the troops are genetically connected and there is no significant genetic differentiation observed. Even between the Boschhoek, Heuningkrans, Schoongezicht and Wolwekloof troops, which are located on the outer boundaries of the reserve, low levels of gene flow are observed and the genetic differentiation is not significant.

CHAPTER THREE
Quality of DNA from non-invasive sampling

3.1 Introduction

A wide range of samples collected non-invasively have been used for genetic analysis, with the use of fecal samples probably being the most popular. A limitation of using DNA isolated from fecal samples is that the DNA is often degraded and isolated in small quantities only (Taberlet *et al*., 1996; Morin *et al*., 2001). The low quality of the isolated DNA may lead to erroneous microsatellite genotyping results because of artefacts or allelic dropout (Piggott *et al*., 2004), due to degradation in primer annealing areas. Since the results from genotyping are commonly used during population studies for the identification of individuals, parentage verification or to determine relatedness between individuals (Constable *et al*., 2001), it is important that such artefacts are avoided. One approach has been to alter PCR methods to reduce genotyping errors and to rely on replications (Taberlet *et al*., 1996; Morin *et al*., 2001). Replication, however, is very time consuming and costly (Piggott *et al*., 2004). The ideal would be to collect fecal samples in such a manner that it contains the optimal amount of DNA needed for accurate genotyping (Taberlet *et al*., 1996; Morin *et al*., 2001). It would then be possible to minimize PCR and genotyping errors by increasing the quality of the DNA, in effect leading to a system based on quality assurance.

The quantity and quality of extracted DNA from fecal samples is influenced by both the initial amount of DNA present in the sample and the amount of DNA present after collection and storage (Nsubuga *et al*., 2004). Factors that will determine the amount of DNA isolated from a fecal sample include the storage technique (Frantzen *et al*., 1998), feeding habits of the animal, physical size of the particular sample, environmental conditions in the collection area (Nsubuga *et al*., 2004) and the time span from defecation to collection (Goossens *et al*., 2000). The interaction of these factors may also lead to different recovery rates of DNA in different species (Frantzen *et al*., 1998). Furthermore,

the use of fecal samples from herbivores presents the additional challenge of minimizing secondary compounds in plants that could inhibit PCR reactions (Fernando *et al*., 2003). Baboons are nominally omnivorous but feed primarily on fruit, leaves, bulbs and other parts of plants, which will result in high concentration secondary compounds in fecal samples. Considering the aforementioned limitations, storage methods and collection strategies used for fecal samples should be optimized to improve the quantity and quality of DNA extracted. However, it is also important that these methods remain practical in the field and that the transport of samples is not subject to any restrictions (Roeder *et al*., 2004).

Frantzen *et al*. (1998) considered the influence of different storage media and the age of baboon fecal samples but there is a need for a comprehensive study where all storage and collection parameters that could influence the quality of genotyping are incorporated, as well as ultimate expression of markers.

3.2 Materials and methods

All fecal samples used for this experiment were collected from a female chacma baboon at the Animal Experimental Unit at the University of the Free State. The animal was kept in a separate enclosure inside the Experimental Unit and no contamination from another baboon's feces was possible. Immediately after defecation, the samples were transferred to a restricted area outside the enclosure where the samples could be exposed to environmental factors without being tampered with. The samples were collected during the month of May - the weather being sunny overall with the exception of two partly cloudy days. The samples were exposed to direct sunlight most of the day. To represent possible time-intervals for sample collection in field studies, samples were collected at seven time intervals. The time intervals were as follow: fresh, 6 hours, 12 hours, 24 hours, 2 days, 4 days and 1 week post defecation. Nine aliquots were taken from each sample using a sterile tongue depressor. Three aliquots were then stored using each of three different storage methods: (i) in sterile tubes filled with silica beads; (ii) in 15 ml sterile tubes containing 10 ml 95 percent (%) ethanol (ETOH); and (iii) using the two-

step method as described by Roeder *et al*. (2004). Upon arrival at the lab the samples were stored at -20˚C and DNA was isolated within a month from the time of collection. Silica beads were replaced when humidity was detected.

In preparation for DNA isolation, approximately 200 grams (g) of fecal material from each sample was cut into smaller pieces with a sterile razor blade. In view of the omnivorous feeding habits of baboons, excessive plant debris was removed in order to prevent PCR inhibition. DNA was then isolated from the fecal samples using the QIAGEN QIAamp® DNA Stool Kit and following the manufacturer's protocol for isolation of DNA from stool for human DNA analysis. The concentration of the extracted DNA was determined using a NanoDrop ND1000 spectrophotometer and by measuring absorbance at 260 nm. The significance of variation between the yields of different storage methods was determined using Analysis of Variance (ANOVA). Approximately 3 ml of whole blood was also collected from the same female baboon as a positive control. DNA isolation of this blood sample was performed using the QIAGEN QIAamp® DNA Mini Kit and quantified using the NanoDrop ND1000 spectrophotometer.

As a measure of DNA quality, and to test whether DNA isolated was in fact baboon DNA, we also amplified eight human microsatellite markers in all isolated DNA samples. The microsatellite loci used were: D1S518, D3S1768, D4S243, D5S1457, D10S611, D11S956, D14S306 and D18S72. The PCR reaction mix was made up of 1x buffer, 10 µM of each primer, 2 mM dNTP's and 2.5 U of Supertherm Taq Gold; and with ddH_2O added to a total volume of 10 µl. The 5' end of the forward primer of each primer-pair was fluorescently labeled. Amplification of DNA was performed using a touch-down protocol. The reaction was started with an initial denaturation step of 12 min at 95˚C. This was followed by 10 cycles of 40 s at 94˚C, 40 s at 58˚C and 1 min at 72˚C; then another 10 cycles followed consisting of 40 s at 94˚C, 40 s at 53˚C and 1 min at 72˚C; and thirdly 20 cycles consisting of 40 s at 94˚C, 40 s at 43˚C and 1 min at 72˚C. A final extension step was performed at 72˚C for 60 min. The PCR products were separated using an ABI 3130 Genetic Analyzer and alleles were sized using GeneMapper™

Software (version 3). Each locus was amplified in blood, fecal samples stored immediately after defecation, and fecal samples collected a week post-defecation, with three repetitions for each locus (=168 reactions).

3.3 Results and discussion

The DNA concentration at each collection interval for each storage method is presented in Table 3.1.

Table 3.1: Average DNA concentration (ng/µl) of baboon fecal samples collected at seven different time intervals and stored using three different methods (with three repetitions in all cases)

Sample age	Storage method		
	Silica	**Two-step method**	**95% ETOH**
Fresh	102.160±80.736	36.213±5.822	36.257±15.412
6 hours	72.217±49.281	28.500±17.563	85.367±36.094
12 hours	28.870±19.754	12.570±5.098	17.333±10.394
24 hours	31.890±34.530	21.337±22.209	6.697±1.008
2 days	56.737±50.254	15.690±13.120	22.027±25.330
4 days	34.217±19.031	69.750±13.976	9.767±11.897
1 week	40.377±19.889	38.347±23.018	10.387±5.799
Average (based on all data):	52.352±45.076	31.772±22.665	26.833±30.593

The amount of DNA present in the extracted samples did not decrease gradually at every time interval as expected. From a quantitative perspective, silica proved to be the best storage method for the baboon fecal samples with the highest average DNA yield of 52.352 ng/µl compared to average yields of 26.833-31.772 ng/µl for the ETOH and two-step methods used. ANOVA analyses (based on all available quantification values) showed that these differences between the three methods were significant with P=0.043. These results differ from trends reported by Roeder *et al*. (2004) who found that storage in ETOH or using the two-step method has a greater DNA yield than storage in silica using gorilla (*Gorilla gorilla*) feces. However, these authors used samples that were collected and stored no later than 5 hours post defecation. It is also possible that species-based and food composition differences might account for differences in the amount of DNA obtained from fecal samples, however further investigation into this matter is

required. Note that DNA concentrations recorded on the NanoDrop ND1000 do not necessarily provide a conclusive indication on the proportion of the total DNA isolate made-up from target-species DNA when using fecal samples. Also, the apparent success of the two-step method is offset by the potential risk of contamination or sample mix-up when transferring samples from ETOH to silica when using the technique (Roeder *et al*., 2004).

The variations in yield between repeat trials of samples of the same sample age stored similarly was very high in many instances, with a coefficient of variation of up to 100% between three runs. Multiple factors can possibly affect results obtained from fecal samples and induce such inconsistencies. Physical differences during sampling, i.e. the area and depth of the fecal sample used for sampling could induce bias depending on the presence or absence of baboon DNA in the specific region sampled. Secondary compounds associated with different diets and time of sample exposure to environmental conditions before collection (together with different habitats) may also account for possible differences in results (Wehausen *et al*., 2004). All eight microsatellite loci used were successfully amplified in the control blood sample. The success of amplification of these fragments after diverse collection and storage regimes are presented in Table 3.2.

Table 3.2: Microsatellite fragments amplified at eight loci, after seven diverse collection and storage regimes. The symbol "0" indicates alleles scored in control samples but lost in others.

	Success rate	**D5S1457**	**D10S611**	**D11S956**	**D4S243**	**D18S72**	**D1S518**	**D14S306**	**D3S1768**
Blood fresh	8/8 (100%)	123/123	190/194	193/193	161/169	182/190	199/199	175/179	201/201
Silica fresh	5/8 (62.5%)	123/123	190/194	193/193	161/169	182/0	- / -	- / -	201/201
Silica 1 week	5/8 (62.5%)	123/123	0/194	193/193	- / -	- / -	199/199	175/179	201/201
95% ETOH fresh	8/8 (100%)	123/123	190/194	193/193	161/169	182/190	199/199	175/179	201/201
95% ETOH 1 week	7/8 (87.5%)	123/123	- / -	193/193	161/169	182/190	199/199	175/179	201/201
Two-step fresh	6/8 (75%)	123/123	190/194	193/193	- / -	- / -	199/199	175/179	201/201
Two-step 1 week	3/8 (37.5%)	123/123	0/194	193/193	- / -	- / -	- / -	- / -	201/201

Samples stored in 95% ETOH provided the most promising results, with 100% of loci successfully amplified when samples were collected fresh and 87.5% loci amplified accurately when collected after 1 week. Success at these two time intervals dropped to 62.5% / 62.5% for samples stored in silica, and 75% / 37.5% for those stored using the two-step procedure. Fragments amplified at locus D3S1768 (with 100% success) are presented in Fig 3.1a. Results for D10S611, where some allelic dropout occurred with delayed collection, are shown in Fig 3.1b.

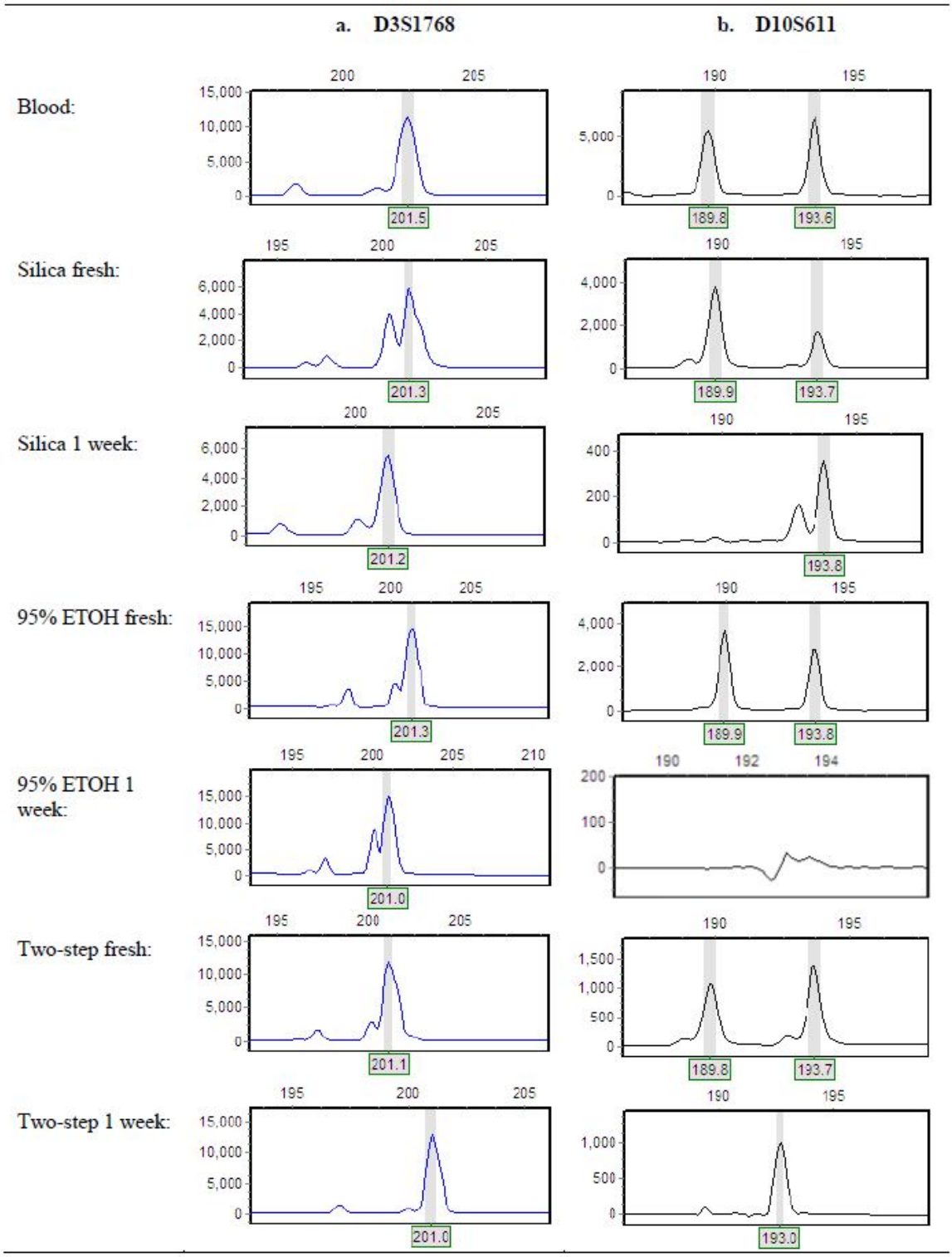

Figure 3.1: Genotypes obtained for D10S611 and D3S1768, using control blood samples and material collected after defecation and 1 week post defecation, and stored according to three methods.

Of concern is that the latter locus (D10S611) as well as locus D18S72 lost specific alleles while still yielding a nominal genotype, which would then be erroneously scored as homozygous in some samples. For example, the genotype 190/194 was scored at D10S611 in blood and fresh fecal samples, but allele 190 was not scored in fecal samples stored for one week in silica or using the two step method. There was no correlation between loci with long fragment length and propensity to degradation over time, since the shortest fragment used (D5S1457 – 123 base pairs (bp)) and the longest (D3S1768 – 201bp) provided 100% repeatable results, whereas fragments of intermediate length did show susceptibility to degradation. It would however be informative to test for the behavior of microsatellites with very long fragments after extended periods of exposure to elements before collection.

Overall, storage in ETOH provided the best results, despite comparatively low DNA concentrations. This confirms that concentrations recorded on the NanoDrop ND1000 do not necessarily provide a reliable indication of the amount of target DNA present in a DNA isolate. The successful amplification of microsatellite loci in fecal samples compared to blood is consistent with results reported by Bayes *et al*. (2000). However, these authors used fecal samples that were collected within minutes of defecation and the results from this study provide new data on the sensitivity of DNA to longer periods before collection and storage. The sensitivity of primers at individual loci affects amplification success, as reported by Vidya & Sukumar (2005), and the number genotypes that could be scored for the fecal samples were indeed significantly lower at some loci than those for the blood sample. The results nevertheless confirm that fecal samples can be used as a reliable source of DNA up to one week post defecation, with suitable collection and storage protocols and if carefully selected loci are used.

CHAPTER FOUR

Individual identification using non-invasive sampling

4.1 Introduction

Counting individuals in a population represents one of the first methods to monitor and manage a population (Gaidet *et al*., 2005) but the size estimates are often difficult to obtain. Individual identification is a critical part of conservation to estimate and monitor the size of a population (Kohn *et al*., 1999), and to estimate the home range of individuals (Taberlet *et al*., 1997) and populations. Individuals are characterized by a unique multilocus genotype and therefore it is possible to determine the number of animals in the population (Bellemain *et al*., 2005).

The number of individuals present in a population and the number of reproductively active individuals are of particular interest to researchers studying primates. This information is critical in the development and implementation of conservation and management strategies (Vigilant & Guschanski, 2009). Field researchers generally rely on individual identification and observations to do a population census (Vigilant & Guschanski, 2009). In cases where direct counts are difficult due a challenging habitat or area size, alternative methods have been considered. Genetic methods are now being used to determine population sizes

Methods based on the collection of non-invasive samples have been developed and collection of samples can take place without disturbing the animals (Taberlet & Luikart, 1999). The difficulty experienced with acquiring an adequate amount of blood or tissue samples for analyses, has been a limiting factor to the application of molecular techniques to field studies. The use of fecal samples provides a promising alternative as this material

is abundant and can be collected non-invasively (Wasser *et al*., 1997). Optimization done for the current study (Chapter 3) demonstrated the utility of microsatellite markers amplified from fecal studies.

4.1.1 Why non-invasive sampling?

Degraded DNA can still be amplified because microsatellites are usually shorter in length (Taberlet *et al*., 1999). Microsatellite markers can provide individuals with unique DNA profiles when more than one marker is used. The possibility of identifying individuals through genotyping following the collection of their fecal samples thus offers the potential to determine population estimates with accuracy and to determine behavioral parameters such as home range size, habitat preferences and diet combination (Piggot & Taylor, 2003).

Non-invasive techniques offer the potential to investigate aspects of a population that are difficult to address through observation alone (Constable *et al*., 2001). The study of primates is widely implemented in the field of molecular ecology but faces a number of challenges. Capturing primates in order to collect morphological data and obtain samples such as blood or tissue can be a difficult task due to their relative intelligence and the areas they inhabit. Non-invasive sampling methods provide a useful tool to overcome this obstacle, however, the limited amounts and the quality of DNA complicates genetic analysis (Vigilant & Guschanski, 2009).

Non-invasive samples such as feces, hair, urine, saliva or remains from food items can be collected in addition to field surveys to census a population (Vigilant & Guschanski, 2009). In addition to population size estimates, genotyping results can also be used to determine other population estimates (Vigilant & Guschanski, 2009). The use of non-invasive samples has created new possibilities for determining the genetic diversity, dispersal patterns and the structure of a population (Ball *et al*., 2007; Fernando *et al*., 2003). The collection of fecal samples has the advantage of not disturbing the animals and offers the potential to study a greater number of individuals in a population than

would have been possible using invasive techniques (Wasser *et al*., 1997; Taberlet *et al*., 1999; Morin *et al*., 2001; Fernando *et al*., 2003; Piggott & Taylor, 2003; Ball *et al*., 2007). Other important advantages of using feces as a source of non-invasive DNA is that it does not require Convention on International Trade in Endangered Species (CITES) permits for international transport, and it can also be used for hormonal tests, dietary information and parasitological analyses (Luikart *et al*., 2008).

4.1.2 Problems associated with non-invasive sampling

Multilocus genotyping makes it possible to identify individuals from highly degraded sources of DNA (Roon *et al*., 2005). As mentioned in Chapter 3, the analysis of fecal samples can be prone to errors, especially when using microsatellites. The most common errors observed when working with non-invasive samples such as fecal samples, is allelic dropout, false alleles or artefacts, and null alleles. Allelic dropout refers to one allele that fails to amplify in a heterozygous individual during PCR. As a result, heterozygotes can be mistakenly identified as homozygotes (Taberlet *et al*., 1996). False alleles are the result of slippage artefacts during the first cycles of PCR (Broquet *et al*., 2007). Null alleles are observed when PCR amplification fails due to PCR conditions that are not ideal or there are mutations in the primer-binding region that inhibits binding. Results for heterozygotes may then be interpreted as homozygotes or the alleles may completely fail to amplify (Selkoe *et al*., 2006). Individual profiles can be used to estimate the size of the population, which presents a risk: if genotyping errors are not identified, the size of the population could be overestimated. Samples collected non-invasively are also known to provide DNA extracts with contamination by foreign DNA (from bacteria and the animal's diet). Species specific markers can be used to avoid amplification of foreign DNA (Bradley & Vigilant, 2002). Amplification and genotyping success can be influenced by the source of DNA, fragment length and repeat motif of microsatellite loci (Broquet *et al*., 2007).

4.1.3 Aim & Objectives

The aim of this part of the study was to determine whether it is possible to use fecal samples collected from a sleeping site of chacma baboons to determine individual profiles that can be compared to reference samples and establish if an individual can be identified using fecal samples. A second aim was to determine if the number of individuals in a troop can be determined using the genetic profiles obtained from fecal samples. In order to ensure maximum DNA quality, an experiment was conducted at the Animal Experimental Unit at the University of the Free State before collection of samples at Suikerbosrand Nature Reserve. The experiment (as described in Chapter 3) involved the analyses of DNA yield for three different methods at seven different time periods after collection. The method which provided the best results was then used for sample collection at Suikerbosrand Nature Reserve.

4.2 Material and Methods

4.2.1 Study population

The sleeping sites of all the troops were identified from the geographic information system (GIS) data collected (See figure 4.1). The Diepkloof troop was chosen as the study population from which fecal samples would be collected. The sleeping site of the Diepkloof troop is located on a cliff. In general, baboons use sleeping sites on a rotational basis. However, the Diepkloof troop provided a unique opportunity because it was the only troop, at the time, using that specific sleeping site in the cliff. Thus, contamination of the sleeping site with fecal samples from other troops was not a problem.

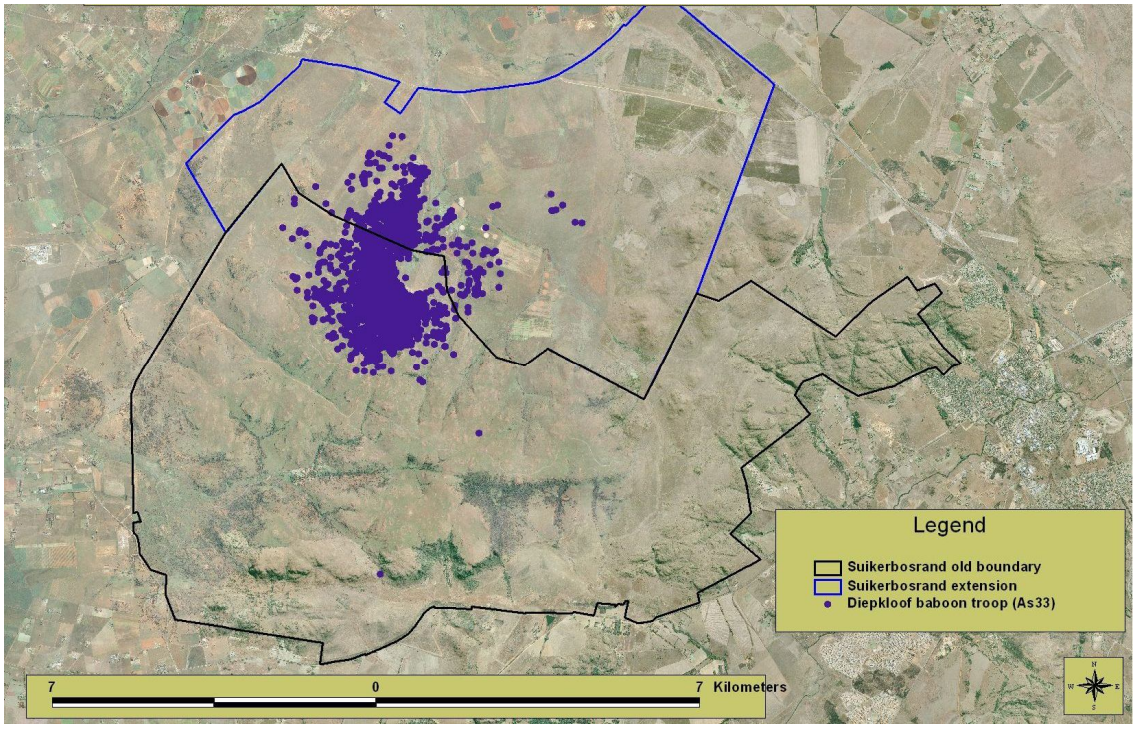

Figure 4.1: GIS data collected for the Diepkloof troop, showing the home range of this troop.

4.2.2 Sample collection

A total of 36 fecal samples were collected in duplicate from the Diepkloof sleeping site over a period of four days. Even though collection in ETOH provided the best results for

genotyping (see Chapter 3), the silica method was used for collection. The reason for the latter is because the silica method is not subject to any restrictions during transport and it still provided adequate results for genotyping and high concentration DNA after extraction. The fecal samples were collected during the winter (July), since fecal samples collected in the winter yields a higher DNA quality than samples collected in other seasons (Piggott & Taylor, 2003). DNA is more likely to be preserved in colder environmental conditions as cell degradation is reduced (Ball *et al.*, 2007). All the samples were estimated to be less than 15 days old upon collection.

4.2.3 DNA extraction

The QIAamp® DNA Stool Kit was used for the extraction of DNA from the fecal samples. The protocol, *Isolation of DNA from Stool for Human DNA Analysis*, was followed according to the manufacturer's instructions with a slight modification in the elution step. The elution step was modified by adding 50 µl of elution buffer instead of 200 µl as set out in the protocol. The concentration of DNA in the eluate is increased if a smaller volume of elution buffer is added.

4.2.4 Determining DNA concentration

The DNA concentration was determined with a NanoDrop ND1000 spectrophotometer by measuring absorbance at 260 nm. The purity of the DNA was determined by calculating the ratio of absorbance at 260 nm (A_{260}) and 280 nm (A_{280}). An A_{260}/A_{280} ratio of ~1.8 is an indication of pure DNA. The DNA concentration of each sample is given in Table 4.1.

Table 4.1: DNA concentration as measured with a NanoDrop ND1000 spectrophotometer for the 36 fecal samples collected at the Diepkloof sleeping site.

Sample number	DNA concentration (ng/µl)
SBR 1	197.9
SBR 2	122
SBR 3	13
SBR 4	96.4
SBR 5	174.7
SBR 6	30.6
SBR 7	42.7
SBR 8	103.7
SBR 9	117.7
SBR 10	163.9
SBR 11	150.8
SBR 12	33.6
SBR 13	67.2
SBR 14	81.6
SBR 15	74.8
SBR 16	91.9
SBR 17	92.7
SBR 18	145.3
SBR 19	65.7
SBR 20	106.7
SBR 21	87.3
SBR 22	83.5
SBR 23	44.7
SBR 24	147.1
SBR 25	54.7
SBR 26	76.9
SBR 27	137.8
SBR 28	77.9

SBR 29	128.5
SBR 30	72.1
SBR 31	309.2
SBR 32	35.6
SBR 33	71.6
SBR 34	130.5
SBR 35	52.3
SBR 36	33.6

4.2.5 PCR-based amplification of fragments

The panel of 11 human microsatellite markers that was selected for the population study was also used for the fecal samples (See Table 2.2).

Supertherm *Taq* DNA polymerase was used for the amplification of the fecal samples because it has a higher specificity and is ideal for applications involving low-copy target DNA and multiple primer pairs. $MgCl_2$ was not considered as an optimization factor as it was already optimized for the Supertherm *Taq* DNA polymerase. The primer concentration was increased to 10 μM for the fecal samples compared to the 1.25 μM for the blood and tissue samples.

4.2.6 Genotyping

Fluorescently labeled fragments were detected using the ABI PRISM® 3130 Genetic Analyzer and the alleles were sized using GeneMapper® software (version 3).

GeneScan™ –LIZ® (Applied Biosystems) was used as an internal size standard for all the primers except for D15S108 (labeled with HEX), in which case GeneScan™ 400HD (ROX™ Dye, Applied Biosystems) was used.

Microsatellite loci were amplified together in plexes as described in Chapter 2. In order to obtain a reliable profile for the sampled individuals, each sample was amplified and genotyped seven times.

4.2.7 Statistical analysis

The probability of identity was determined using GIMLET software. GIMLET (Genetic Identification with Multilocus Tags), developed by Valière (2002), is a computer program designed for the identification of identical genotypes, comparison of new genotypes to a set of reference genotypes, and the estimation of population parameters such as probability of identity.

4.3 Results and Discussion

DNA was extracted successfully from all the fecal samples. The modification of the elution step in the extraction protocol significantly increased the DNA concentration of the samples.

The T_a for the selected primers was generally lower for the fecal samples than for the blood and tissue samples. Optimization for the T_a started at 48˚C. The temperature was then adjusted until the required level of specificity was obtained. The higher the T_a, the more specific the reaction became. The temperature was increased to enhance the specificity and reduce background peaks.

The sensitivity of primers at individual loci affects amplification success (Fernando *et al*., 2003; Vidya & Sukumar, 2005) and because the extracted DNA was generally low in quality, a high level of sensitivity was required in order to detect the lower peaks.

Amplification success of the samples decreased the older the samples were. There was also a differential decrease in amplification success across loci. This is a result of variation in primer sensitivity.

4.3.1 Genotyping

The above mentioned problems that are associated with the amplification of DNA from fecal samples are now well understood and errors in genotyping results can be limited (Bellemain & Taberlet, 2004). Amplification of all the samples was repeated 5-7 times to account for false alleles and allelic dropout (Taberlet *et al*., 1996). The number of times amplification has to be repeated is likely to be locus specific (Bayes *et al*., 2000). A consensus multi-locus genotype was constructed for each individual from the results of these replications (See Appendix D).

4.3.2 Statistical analysis

4.3.2.1 Probability of identity

The probability of identity (PI) is an estimator for individual identification and it is defined as the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Waits *et al*., 2001).

The PI value is computed using the allele frequencies and the following equations:

- Biased estimation of PI
- Unbiased estimation of PI (for small sample size)
- PI for sibs (Waits *et al*., 2001)

The biased estimation of PI does not correct for sample size whereas the unbiased PI is calculated with a sample size correction. The PI-value is calculated for each locus (Table 4.2) and over several loci (Table 4.3). The PI-value is commonly used to quantify the ability of a marker to resolve between two individuals, and therefore the loci can be sorted by their PI values.

Table 4.2: Probability of identity per locus for the Diepkloof troop

Locus	PI_{biased}	$PI_{unbiased}$	PI_{sibs}
D5S1457	1.179e-01	1.008e-01	4.184e-01
D10S611	1.082e-01	9.338e-02	4.055e-01
D11S956	1.000e+00	1.000e+00	1.000e+00
D4S243	2.897e-01	2.770e-01	5.441e-01
D18S72	2.328e-01	2.157e-01	5.107e-01
D1S518	2.444e-01	2.311e-01	5.114e-01
D14S306	7.738e-02	6.462e-02	3.742e-01
D3S1768	1.368e-01	1.219e-01	4.299e-01
D15S508	5.602e-01	5.393e-01	7.512e-01
D2S1326	3.965e-01	3.745e-01	6.405e-01
D7S2204	3.211e-01	3.109e-01	5.625e-01

Table 4.3: Multi-loci PI in increasing order of single-locus values (the first locus is the most informative locus)

Locus	Prod(biased)	Prod(unbiased)	Prod(sibs)
D14S306	7.738e-02	6.462e-02	3.742e-01
D10S611	8.369e-03	6.034e-03	1.517e-01
D5S1457	9.864e-04	6.083e-04	6.348e-02
D3S1768	1.350e-04	7.416e-05	2.729e-02
D18S72	3.143e-05	1.600e-05	1.394e-02
D1S518	7.683e-06	3.697e-06	7.127e-03
D4S243	2.225e-06	1.024e-06	3.878e-03
D7S2204	7.146e-07	3.184e-07	2.181e-03
D2S1326	2.834e-07	1.192e-07	1.397e-03
D15S508	1.587e-07	6.431e-08	1.049e-03
D11S956	1.587e-07	6.431e-08	1.049e-03

The PI_{sibs}- value needs to be between 0.001 and 0.0001 in order to successfully identify individuals. As indicated in Table 4.3, a PI_{sibs}- value of 0.001, calculated over all the loci, was achieved. Thus, the 11 loci selected were sufficient for individual identification.

Among loci, locus D14S306 and locus D10S611 was the most informative markers for identification and D11S956 the least informative.

4.3.2.2 Comparison of samples collected at the Diepkloof sleeping site

The thirty six fecal samples collected at the sleeping site were compared with each other to establish the unique individual profiles. The following genotypes were identified as one individual:

- SBR 11, SBR 35 and SBR 5 were identified as the same individual. SBR 11 had 12 alleles in common with SBR 5 at 6/11 loci. SBR 35 had 16 alleles in common with SBR 5 at 8/11 loci. (See Table 4.4)

- SBR 16 had 22 alleles in common with SBR 30 at 11/11 loci. (See Table 4.4)

- SBR 14 had 20 alleles in common with SBR 21 at 10/10 loci. D2S1326 did not provide any results for either the samples. (See Table 4.4)

- SBR 20, SBR 22 and SBR 24 were identified as the same individual. SBR 20 had 20 alleles in common with SBR 22 at 10/11 loci. SBR 24 had 20 alleles in common with SBR 22 at 10/11 loci. (See Table 4.4)

Table 4.4: Identical genotypes identified from the 36 fecal samples collected.

SBR	D5S1457		D10S611		D11S956		D4S243		D18S72		D1S518		D14S306		D3S1768		D15S508		D2S1326		D7S2204	
11	123	127	~	~	193	193	157	161	186	186	199	203	~	~	~	~	166	174	~	~	~	~
35	123	127	210	214	193	193	157	161	186	186	199	203	~	~	~	~	166	174	~	~	238	242
5	123	127	210	214	193	193	157	161	186	186	199	203	171	175	185	189	166	174	261	261	238	242
16	123	127	210	210	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
30	123	127	210	210	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
14	123	127	186	186	193	193	157	161	186	190	199	207	163	171	197	201	166	166	~	~	238	242
21	123	127	186	186	193	193	157	161	186	190	199	207	163	171	197	201	166	166	~	~	238	242
20	123	133	202	206	193	193	157	161	186	190	199	203	167	167	185	201	166	166	~	~	238	242
22	123	133	202	206	193	193	157	161	186	190	199	203	167	167	185	201	166	166	261	261	238	242
24	123	133	202	206	193	193	157	161	186	190	199	203	167	167	185	201	166	166	~	~	238	242

4.3.2.3 Comparison of samples collected at sleeping site with reference samples

The genotypes from the fecal samples collected at the Diepkloof sleeping site was compared with the reference genotypes obtained from the blood samples. SBR 16 and SBR 30 (which was identified as one individual in section 4.3.5.2) had the same genotype as BO 81, and SBR 32 had the same genotype as BO 83. Thus, fecal samples were collected from two individuals that were also sampled through capturing (See Table 4.5).

Twenty four genotypes from the fecal samples were identified as unique. SBR 8, SBR 26, SBR 29 and SBR 31 were not taken into account for the estimation of the troop size as there was no amplification for more than half of the loci and an accurate genotype could not be identified (See Appendix D). Thirteen blood samples were also collected from the Diepkloof troop. The minimum estimated size of the Diepkloof troop is thus thirty seven individuals.

The objective of this part of the study was to determine whether fecal samples could be used to estimate the size of the population non-invasively. Two individuals out of the thirteen individuals that were captured and sampled were identified through the genetic profiles constructed from their fecal samples. Thus, the use of fecal samples provides a promising alternative for future research. However, it is recommended that this research is conducted with careful planning with regard to the time of collection (summer or winter), the period of collection (in order to insure maximum variety in the number of collected samples), and the area of collection.

Table 4.5: Genotypes of the fecal samples collected that matched the genotypes from the reference samples

Number	D5S1457		D10S611		D11S956		D4S243		D18S72		D1S518		D14S306		D3S1768		D15S108		D2S1326		D7S2204	
BO81	123	127	210	222	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
BO83	127	127	210	214	193	193	157	161	182	186	203	203	171	175	185	189	166	174	261	261	238	242
SBR16	123	127	210	210	193	193	161	161	190	190	199	207	159	175	185	201	166	166	261	261	242	242
SBR30	123	127	210	210	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
SBR32	127	127	210	214	193	193	157	161	182	186	203	203	171	175	185	189	166	174	261	261	238	242

CHAPTER FIVE

Management implications for Suikerbosrand Nature Reserve

The Suikerbosrand chacma baboon population has been minimally managed as a result of limited information previously available. No genetic study has been conducted on the population before. Genetic management is essential to maintain diversity and avoid inbreeding in the population. In doing so, the long term viability of the population can be ensured.

All the objectives for this study were reached with great success. Genetic profiles were obtained for each of the 145 individuals (out of 14 troops) from which blood samples were collected. Together with the data collected by the Suikerbosrand field team (age, gender, collar ID, etc.), a reference database could be constructed.

Genetic relationships were estimated in order to determine the genetic status of the population. Before any statistical analyses were performed, the microsatellite markers selected for the study were tested for linkage disequilibrium. Even though loci D5S1457 and D1S518; D10S611 and D14S306; and D15S108 and D2S1326 were linked, all the markers were included in the statistical analyses.

The Suikerbosrand population were compared to an outgroup population for genetic differentiation. The genetic differentiation among the two populations was 11%. The differentiation between the troops in Suikerbosrand contributed to 6% of the genetic diversity and the differentiation within troops 83%.

As an additional measure of the genetic connectivity between troops, pair-wise F_{ST} values were calculated. No significant genetic differentiation was observed between the Suikerbosrand troops, or between the Suikerbosrand troops and the outgroup.

In addition to the F_{ST} values, R_{ST} values were calculated. The R_{ST} values also indicated no significant genetic differentiation between any of the troops. The gene flow between the troops was calculated and high levels of gene flow were displayed. High levels of gene flow were observed between the troops located in the centre of the reserve. These include Bezuidenhoutshoek, Groot Plato's, Kareekloof, Wolwekloof and Wetter. There are no barriers to gene flow between any of the fourteen troops. The lowest level of gene flow is observed between the Schikfontein and Toringkop troops.

The observed heterozygosity between the outgroup and the Suikerbosrand population did not indicate a significant difference between the two populations. Results from this study show that the level of genetic diversity in the Suikerbosrand population is relatively high and the introduction of new animals into the population in order to avoid inbreeding and increase genetic diversity, is currently not a priority. The Heuningkrans troop displays the highest genetic diversity and unique alleles were detected in the Heuningkrans and Schikfontein troops. The high genetic diversity and unique alleles observed in the Heuningkrans troop can be attributed to the fact that the troop is located at the outer most boundary of the reserve and contact with other troops might be minimal.

Very few departures from Hardy Weinberg equilibrium ($P<0.05$) were observed at loci D1S518, D2S1326, D3S1768, D4S243, D5S1457, D14S306 and D15S108 and it can be concluded that processes such as non-random mating and selection does not play a significant role in the Suikerbosrand troops.

Fecal samples were collected from the Diepkloof sleeping site with the objectives of identifying individuals and estimating the troop size. From the thirty six samples collected at the sleeping site, twenty four profiles were identified as unique and two of the profiles corresponded with profiles obtained in the reference database. The Diepkloof

troop was estimated to consist of thirty seven individuals. The collection of fecal samples for identification purposes and population size estimates proves to be very promising and can further add to the construction of the genetic database. However, it is recommended that fecal samples are collected over a longer period to ensure that samples can be collected from all the individuals in that specific troop. The collection of fresher samples may also improve the results obtained for the construction of genetic profiles.

Continued sampling and genetic profiling must be conducted to obtain more genetic profiles and maintain an updated genetic database. It is advisable that a genetic database be compiled for all the individuals in the population. The genetic database can aid in the process of monitoring the population size. It is essential to ensure that the biodiversity of the reserve is maintained and that the size of the baboon population does not affect ecological processes, as the carrying capacity of the reserve is decreasing due to developments around the reserve. The total size of the population can be monitored through the non-invasive collection of fecal samples and individuals can be identified from areas where the baboons are causing problems to neighboring land. As a result, management objectives can be formulated, focusing on individuals or specific troops. Since unique alleles have been identified in two of the troops, it is recommended that problems animals be identified according to their genetic profile before serious interventions such as culling can take place.

Management of the Suikerbosrand baboon population is essential to preserve genetic diversity. In view of the data obtained in this study, the management of the chacma baboon population can be improved. The use of molecular methods to establish genetic profiles is a valuable tool for effective management. Though the genetic data offer important insights for the management of the population, it is recommended that management strategies be based on observational information as well. The genetic data collected can be combined with ecological and behavioral information to further understand the population structure and changes that might occur in the population.

SUMMARY

Genetic management has become a critical part of the overall management of nonhuman primate populations. This dissertation describes a genetic analysis of the chacma baboon population at the Suikerbosrand Nature Reserve. The aim of this study was to apply genetic data as a credible tool to contribute to the conservation and management of chacma baboons at Suikerbosrand Nature Reserve. The specific objectives included individual identification, determining genetic relationships and levels of gene flow within- and among the fourteen troops, and to construct a genetic database with individual genotypes of the whole population. A secondary objective of this study was to determine whether it would be feasible to extract DNA from fecal samples collected from a sleeping site and then use the genetic profiles to determine the number of individuals in that specific troop. The current population is estimated to be between 611 and 764 animals. The sleeping site of the Diepkloof troop was used for this part of the study. A panel of eleven human microsatellite markers was used for DNA analysis. DNA profiles from all the blood samples were successfully constructed and could be used to estimate genetic relationships. The level of genetic diversity in the Suikerbosrand baboon population did not differ significantly from that in the outgroup. Thus, the reintroduction of new individuals into the population to maintain acceptable levels of diversity is not an immediate priority. High levels of gene flow were observed between the troops, especially the troops located in the central part of the reserve. In order to ensure high DNA quality from fecal samples collected at the sleeping site, the collection method for fecal samples were optimized (A manuscript based on the work in this section has been accepted for publication in the European Journal of Wildlife Research). The profiles obtained from the fecal samples that were collected at the Diepkloof site corresponded with two of the thirteen profiles from the reference database. The estimated size of the Diepkloof troop is thirty seven individuals. The results show that non-invasive sampling could be a promising alternative for future research on the reserve, as the samples can be used to determine individual profiles. The genetic data collected can be combined with ecological and behavioral information collected form future research to further

understand the population structure of the Suikerbosrand chacma baboons and changes that might occur in the population.

Key words: Suikerbosrand, genetic management, chacma baboons, human microsatellite markers, genetic relationships, gene flow, non-invasive sampling, individual identification, fecal samples, troop size

OPSOMMING

Genetiese bestuur het ‘n kritiese deel geword van die algehele bestuur van nie-menslike primaat populasies. Hierdie verhandeling beskryf ‘n genetiese analise van die Kaapse bobbejaan populasie in die Suikerbosrand Natuurreservaat. Die doel van hierdie studie was om genetiese data toe te pas as ‘n geloofwaardige hulpmiddel om by te dra tot die bewaring en bestuur van Kaapse bobbejane in Suikerbosrand Natuurreservaat. Die spesifieke uitkomste het individuele identifikasie, bepaling van genetiese verwantskappe en vlakke van geenvloei in en tussen die veertien troppe, en die samestelling van ‘n genetiese databasis van die populasie ingesluit. ‘n Sekondêre uitkoms van die studie was om te bepaal of dit moontlik sal wees om DNS te ekstraheer vanuit fekale monsters wat versamel is by ‘n slaapplek, en dan die genetiese profiele te gebruik om die aantal individue in daardie spesifieke trop bepaal. Die huidige populasie word geskat tussen 611 en 764 diere. Die slaapplek van die Diepkloof trop is gebruik vir hierdie deel van die studie. ‘n Paneel van elf menslike mikrosatelliet merkers is gebruik vir die DNS analise. DNS profiele van al die bloedmonsters is suksesvol saamgestel en kon gebruik word om genetiese verwantskappe te bepaal. Die vlak van genetiese diversiteit in die Suikerbosrand bobbejaan populasie het nie betekenisvol verskil van die buitegroep nie. Dus is dit nie ‘n onmiddelike prioriteit om nuwe individue in die populasie in te bring en sodoende aanvaarbare vlakke van diversiteit te onderhou nie. Hoë vlakke van geenvloei is waargeneem tussen die troppe, veral tussen die troppe wat in die sentrale deel van die reservaat geleë is. Om hoë DNS kwaliteit te verseker vanaf die fekale monsters wat by die slaapplek versamel is, is die versamelingsmetode vir fekale monsters geoptimaliseer (‘n Manuskrip gebaseer op die werk in hierdie afdeling is aanvaar vir publikasie in die European Journal of Wildlife Research). Die profiele wat verkry is vanaf die fekale monster wat versamel is in die Diepkloof gebied, het ooreengestem met twee van die dertien profiele uit die databasis van die bloedmonsters. Die geskatte grootte van die Diepkloof trop is sewe-en-dertig individue. Die resultate toon dat nie-indringende versameling van monsters ‘n belowende alternatief vir toekomstige navorsing op die reservaat kan wees, en dat die monsters gebruik kan word om individuele profiele te

bepaal. Die genetiese data wat versamel is kan in die toekoms gekombineer word met ekologiese en gedragsinligting om die populasiestruktuur van die Kaapse bobbejane in Suikerbosrand en veranderinge wat in die populasie mag plaasvind, verder te verstaan.

Sleutelwoorde: Suikerbosrand, genetiese bestuur, Kaapse bobbejane, menslike mikrosatelliet merkers, genetiese verwantskappe, geenvloei, nie-indringende monsterneming, individuele identifikasie, fekale monsters, tropgrootte

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APPENDIX A

Sample list: Suikerbosrand baboon population

NO	SAMPLE	SPECIES	COLLAR ID	SEX	AGE	SAMPLE TYPE	ORIGIN	LOCATION
1	BO1/05	Chacma baboon	1 yellow	F	sub adult	blood	Suikerbosrand	Diepkloof
2	BO2/05	Chacma baboon	2 yellow	F	adult	blood	Suikerbosrand	Diepkloof
3	BO3/05	Chacma baboon	3 yellow	M	sub adult	blood	Suikerbosrand	Diepkloof
4	BO4/05	Chacma baboon	4 yellow	M	adult	blood	Suikerbosrand	Diepkloof
5	BO5/05	Chacma baboon	5 yellow	M	sub adult	blood	Suikerbosrand	Diepkloof
6	BO6/05	Chacma baboon	cell yellow	F	adult	blood	Suikerbosrand	Diepkloof
7	BO7/05	Chacma baboon	orange 1	M	sub adult	blood	Suikerbosrand	Boschhoek
8	BO8/05	Chacma baboon	orange 2	M	adult	blood	Suikerbosrand	Boschhoek
9	BO9/05	Chacma baboon	orange 3	F	adult	blood	Suikerbosrand	Boschhoek
10	BO10/05	Chacma baboon	orange 4	F	adult	blood	Suikerbosrand	Boschhoek
11	BO11/05	Chacma baboon	orange 5	F	adult	blood	Suikerbosrand	Boschhoek
12	BO12/05	Chacma baboon	cell orange	F	adult	blood	Suikerbosrand	Boschhoek
13	BO13/05	Chacma baboon	blue 1	M	sub adult	blood	Suikerbosrand	Heuningkrans
14	BO14/05	Chacma baboon	blue 2	F	sub adult	blood	Suikerbosrand	Heuningkrans
15	BO15/05	Chacma baboon	blue 3	F	adult	blood	Suikerbosrand	Heuningkrans
16	BO16/05	Chacma baboon	blue 4	F	sub adult	blood	Suikerbosrand	Heuningkrans
17	BO17/05	Chacma baboon	blue 5	M	adult	blood	Suikerbosrand	Heuningkrans
18	BO18/05	Chacma baboon	blue 7	M	adult	blood	Suikerbosrand	Heuningkrans
19	BO19/05	Chacma baboon	cell blue	F	adult	hair	Suikerbosrand	Heuningkrans
20	BO20/05	Chacma baboon	red 1	M	adult	blood	Suikerbosrand	Kareekloof
21	BO21/05	Chacma baboon	red 2	M	sub adult	blood	Suikerbosrand	Kareekloof
22	BO22/05	Chacma baboon	red 3	M	adult	blood	Suikerbosrand	Kareekloof
23	BO23/05	Chacma baboon	red 4	F	sub adult	blood	Suikerbosrand	Kareekloof
24	BO24/05	Chacma baboon	red 5	M	sub adult	blood	Suikerbosrand	Kareekloof
25	BO25/05	Chacma baboon	red 6	F	adult	blood	Suikerbosrand	Kareekloof
26	BO26/05	Chacma baboon	red 7 (no collar)	M	adult	blood	Suikerbosrand	Kareekloof
27	BO27/05	Chacma baboon	cell red	F	adult	blood	Suikerbosrand	Kareekloof
28	BO28/05	Chacma baboon	orange & black 1	F	adult	blood	Suikerbosrand	Schoongezicht
29	BO29/05	Chacma baboon	orange & black 2	M	adult	blood	Suikerbosrand	Schoongezicht
30	BO30/05	Chacma baboon	orange & black 3	M	adult	blood	Suikerbosrand	Schoongezicht
31	BO31/05	Chacma baboon	orange & black 4	F	adult	blood	Suikerbosrand	Schoongezicht
32	BO32/05	Chacma baboon	orange & black 5	M	adult	blood	Suikerbosrand	Schoongezicht
33	BO33/05	Chacma baboon	orange & black cell	F	adult	blood	Suikerbosrand	Schoongezicht
34	BO34/05	Chacma baboon	blue & yellow 1	F	adult	hair	Suikerbosrand	Steenbokhut
35	BO35/05	Chacma baboon	blue & yellow 2	F	adult	blood	Suikerbosrand	Steenbokhut
36	BO36/05	Chacma baboon	blue & yellow 4	M	sub adult	blood	Suikerbosrand	Steenbokhut
37	BO37/05	Chacma baboon	blue & yellow 5	F	adult	blood	Suikerbosrand	Steenbokhut
38	BO38/05	Chacma baboon	blue & yellow cell	F	adult	blood	Suikerbosrand	Steenbokhut
39	BO39/05	Chacma baboon	yellow 1	M	sub adult	blood	Suikerbosrand	Wetter
40	BO40/05	Chacma baboon	yellow 2	F	adult	blood	Suikerbosrand	Wetter
41	BO41/05	Chacma baboon	yellow 3	M	sub adult	blood	Suikerbosrand	Wetter
42	BO42/05	Chacma baboon	yellow 5	F	adult	blood	Suikerbosrand	Wetter
43	BO43/05	Chacma baboon	yellow 6 (no collar)	M	adult	blood	Suikerbosrand	Wetter
44	BO44/05	Chacma baboon	yellow cell	F	adult	blood	Suikerbosrand	Wetter
45	BO45/05	Chacma baboon	grey 1	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek
46	BO46/05	Chacma baboon	grey 2	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek
47	BO47/05	Chacma baboon	grey 3	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek
48	BO48/05	Chacma baboon	grey 4	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek
49	BO49/05	Chacma baboon	grey 5	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek
50	BO50/05	Chacma baboon	grey 6	M	sub adult	blood	Suikerbosrand	Bezuidenhoutshoek
51	BO51/05	Chacma baboon	grey cell	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek
52	BO52/05	Chacma baboon	blue-w 1	F	adult	blood	Suikerbosrand	Wolwekloof
53	BO53/05	Chacma baboon	blue-w 2	M	adult	blood	Suikerbosrand	Wolwekloof
54	BO54/05	Chacma baboon	blue-w 3	M	sub adult	blood	Suikerbosrand	Wolwekloof
55	BO55/05	Chacma baboon	blue-w 4	M	adult	blood	Suikerbosrand	Wolwekloof
56	BO56/05	Chacma baboon	blue-w 5	M	adult	blood	Suikerbosrand	Wolwekloof
57	BO57/05	Chacma baboon	cell blue-w	F	adult	blood	Suikerbosrand	Wolwekloof

58	BO58/05	Chacma baboon	1 red + black	M	adult	blood	Suikerbosrand	Feeshuis
59	BO59/05	Chacma baboon	2 red + black	F	adult	blood	Suikerbosrand	Feeshuis
60	BO60/05	Chacma baboon	3 red + black	F	adult	blood	Suikerbosrand	Feeshuis
61	BO61/05	Chacma baboon	cell red + black	F	adult	blood	Suikerbosrand	Feeshuis
62	BO62/05	Chacma baboon	orange v 1	M	adult	blood	Suikerbosrand	Valsfontein
63	BO63/05	Chacma baboon	orange v 2	F	adult	blood	Suikerbosrand	Valsfontein
64	BO64/05	Chacma baboon	orange v 3	M	adult	blood	Suikerbosrand	Valsfontein
65	BO65/05	Chacma baboon	orange v 4	F	adult	blood	Suikerbosrand	Valsfontein
66	BO66/05	Chacma baboon	orange v 5	F	adult	blood	Suikerbosrand	Valsfontein
67	BO67/05	Chacma baboon	orange v cell	F	adult	blood	Suikerbosrand	Valsfontein
68	BO68/05	Chacma baboon	brown 1	F	adult	blood	Suikerbosrand	Groot Platos
69	BO69/05	Chacma baboon	brown 2	F	adult	blood	Suikerbosrand	Groot Platos
70	BO70/05	Chacma baboon	brown 3	F	adult	blood	Suikerbosrand	Groot Platos
71	BO71/05	Chacma baboon	brown 4	M	adult	blood	Suikerbosrand	Groot Platos
72	BO72/05	Chacma baboon	brown 5	F	adult	blood	Suikerbosrand	Groot Platos
73	BO73/05	Chacma baboon	brown 6 (no collar)	M	sub adult	blood	Suikerbosrand	Groot Platos
74	BO74/05	Chacma baboon	brown cell	F	adult	blood	Suikerbosrand	Groot Platos
75	BO75/06	Chacma baboon	6 red	M	sub adult	blood	Suikerbosrand	Feeshuis
76	BO76/06	Chacma baboon	7 red	F	adult	blood	Suikerbosrand	Feeshuis
77	BO77/06	Chacma baboon	8 red	F	adult	blood	Suikerbosrand	Feeshuis
78	BO78/06	Chacma baboon	cell	F	adult	blood	Suikerbosrand	Feeshuis
79	BO79/07	Chacma baboon	D12	F	adult	blood	Suikerbosrand	Diepkloof
80	BO80/07	Chacma baboon	D7	M	adult	blood	Suikerbosrand	Diepkloof
81	BO81/07	Chacma baboon	D11	F	adult	blood	Suikerbosrand	Diepkloof
82	BO82/07	Chacma baboon	D10	M	adult	blood	Suikerbosrand	Diepkloof
83	BO83/07	Chacma baboon	D9	M	sub adult	blood	Suikerbosrand	Diepkloof
84	BO84/07	Chacma baboon	D8	M	sub adult	blood	Suikerbosrand	Diepkloof
85	BO85/07	Chacma baboon	D13	F	adult	blood	Suikerbosrand	Diepkloof
86	BO86/07	Chacma baboon	8 (a)	F	adult	blood	Suikerbosrand	Wolwekloof
87	BO87/07	Chacma baboon	9 (a)	M	sub adult	blood	Suikerbosrand	Wolwekloof
88	BO88/07	Chacma baboon	10 (a)	F	adult	blood	Suikerbosrand	Wolwekloof
89	BO89/07	Chacma baboon	11 (a)	F	adult	blood	Suikerbosrand	Wolwekloof
90	BO90/07	Chacma baboon	12 (a)	F	adult	blood	Suikerbosrand	Wolwekloof
91	BO91/07	Chacma baboon	7 (a)	F	adult	tissue	Suikerbosrand	Wolwekloof
92	BO92/07	Chacma baboon	7 (b)	M	adult	blood	Suikerbosrand	Heuningkrans
93	BO93/07	Chacma baboon	8 (b)	F	adult	blood	Suikerbosrand	Heuningkrans
94	BO94/07	Chacma baboon	9 (b)	M	juvenile	blood	Suikerbosrand	Heuningkrans
95	BO95/07	Chacma baboon	12 (b)	M	juvenile	blood	Suikerbosrand	Heuningkrans
96	BO96/07	Chacma baboon	10 (b)	F	adult	blood	Suikerbosrand	Heuningkrans
97	BO97/07	Chacma baboon	11 (b)	F	adult	blood	Suikerbosrand	Heuningkrans
98	BO98/07	Chacma baboon	7 (c)	M	adult	blood	Suikerbosrand	Boschhoek
99	BO99/07	Chacma baboon	8 (c)	F	adult	blood	Suikerbosrand	Boschhoek
100	BO100/07	Chacma baboon	9 (c)	M	adult	blood	Suikerbosrand	Boschhoek
101	BO101/07	Chacma baboon	10 (c)	F	adult	blood	Suikerbosrand	Boschhoek
102	BO102/07	Chacma baboon	11 (c)	F	adult	tissue	Suikerbosrand	Boschhoek
103	BO103/07	Chacma baboon	12 (c)	M	sub adult	blood	Suikerbosrand	Boschhoek
104	BO104/07	Chacma baboon	13 (c)	M	adult	blood	Suikerbosrand	Boschhoek
105	BO105/07	Chacma baboon	14 (c)	F	adult	tissue	Suikerbosrand	Boschhoek
106	BO106/07	Chacma baboon	15 (c)	M	adult	blood	Suikerbosrand	Boschhoek
107	BO107/07	Chacma baboon	7 (d)	F	adult	blood	Suikerbosrand	Schoongezicht
108	BO108/07	Chacma baboon	8 (d)	F	adult	blood	Suikerbosrand	Schoongezicht
109	BO109/07	Chacma baboon	9 (d)	F	adult	blood	Suikerbosrand	Schoongezicht
110	BO110/07	Chacma baboon	10 (d)	F	adult	blood	Suikerbosrand	Schoongezicht
111	BO111/07	Chacma baboon	11 (d)	M	adult	tissue	Suikerbosrand	Schoongezicht
112	BO112/07	Chacma baboon	12 (d)	F	adult	tissue	Suikerbosrand	Schoongezicht
113	BO113/07	Chacma baboon	7 (e)	M	sub adult	blood	Suikerbosrand	Steenbokhut
114	BO114/07	Chacma baboon	8 (e)	F	adult	tissue	Suikerbosrand	Steenbokhut
115	BO115/07	Chacma baboon	9 (e)	F	adult	tissue	Suikerbosrand	Steenbokhut

116	BO116/07	Chacma baboon	10 (e)	M	adult	blood	Suikerbosrand	Steenbokhut
117	BO117/07	Chacma baboon	11 (e)	M	adult	blood	Suikerbosrand	Steenbokhut
118	BO118/07	Chacma baboon	12 (e)	F	adult	blood	Suikerbosrand	Steenbokhut
119	BO01/08	Chacma baboon	1	F	adult	blood	Suikerbosrand	Kareekloof (AS 39)
120	BO02/08	Chacma baboon	2	M	adult	blood	Suikerbosrand	Kareekloof (AS 39)
121	BO03/08	Chacma baboon	3	F	adult	blood	Suikerbosrand	Kareekloof (AS 39)
122	BO04/08	Chacma baboon	4	F	adult	blood	Suikerbosrand	Kareekloof (AS 39)
123	BO05/08	Chacma baboon	5	F	adult	blood	Suikerbosrand	Kareekloof (AS 39)
124	BO06/08	Chacma baboon	6	F	adult	blood	Suikerbosrand	Kareekloof (AS 39)
125	BO07/08	Chacma baboon	1A A	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek (AS 56)
126	BO08/08	Chacma baboon	1A B	F	sub adult	blood	Suikerbosrand	Bezuidenhoutshoek (AS 56)
127	BO09/08	Chacma baboon	2A	M	sub adult	blood	Suikerbosrand	Bezuidenhoutshoek (AS 56)
128	BO10/08	Chacma baboon	3A	F	sub adult	blood	Suikerbosrand	Bezuidenhoutshoek (AS 56)
129	BO11/08	Chacma baboon	4A	F	adult	blood	Suikerbosrand	Bezuidenhoutshoek (AS 56)
130	BO12/08	Chacma baboon	1B	F	adult	blood	Suikerbosrand	Feeshuis (AS44)
131	BO13/08	Chacma baboon	2B	M	adult	blood	Suikerbosrand	Feeshuis (AS44)
132	BO14/08	Chacma baboon	3B	F	sub adult	blood	Suikerbosrand	Feeshuis (AS44)
133	BO15/08	Chacma baboon	4B	M	sub adult	tissue	Suikerbosrand	Feeshuis (AS44)
134	BO16/08	Chacma baboon	5B	F	sub adult	blood	Suikerbosrand	Feeshuis (AS44)
135	BO17/08	Chacma baboon	6B	M	sub adult	blood	Suikerbosrand	Feeshuis (AS44)
136	BO18/08	Chacma baboon	1C	M	sub adult	blood	Suikerbosrand	Schikfontein (AS36)
137	BO19/08	Chacma baboon	2C	F	sub adult	tissue	Suikerbosrand	Schikfontein (AS36)
138	BO20/08	Chacma baboon	3C	F	adult	tissue	Suikerbosrand	Schikfontein (AS36)
139	BO21/08	Chacma baboon	4C	M	adult	blood	Suikerbosrand	Schikfontein (AS36)
140	BO01/09	Chacma baboon	1	F	adult	blood	Suikerbosrand	Toringkop
141	BO02/09	Chacma baboon	2	F	adult	blood	Suikerbosrand	Toringkop
142	BO03/09	Chacma baboon	3	F	adult	blood	Suikerbosrand	Toringkop
143	BO04/09	Chacma baboon	4	F	adult	blood	Suikerbosrand	Toringkop
144	BO05/09	Chacma baboon	5	M	sub adult	blood	Suikerbosrand	Toringkop
145	BO06/09	Chacma baboon	6	M	sub adult	blood	Suikerbosrand	Toringkop

APPENDIX B

Genetic profiles: Suikerbosrand baboon population

NO	LAB NO	D5S1457		D10S611		D11S956		D4S243		D18S72		D1S518		D14S306		D3S1768		D15S108		D2S1326		D7S2204	
1	BO1/05	123	125	186	206	193	193	165	165	182	190	191	203	163	171	185	197	166	166	249	261	238	254
2	BO2/05	123	123	206	206	193	193	157	165	182	186	191	203	171	175	185	197	166	166	249	261	254	254
3	BO3/05	125	127	214	222	193	193	157	165	190	190	203	203	171	175	185	197	166	166	261	261	238	238
4	BO4/05	127	137	210	222	193	193	157	161	186	190	191	191	171	175	189	189	166	166	237	253	238	242
5	BO5/05	123	127	210	210	193	193	161	165	182	186	203	203	159	171	189	197	166	166	257	261	238	250
6	BO6/05	127	137	206	222	193	193	157	165	186	186	203	207	163	175	197	201	166	174	237	261	242	246
7	BO7/05	123	125	190	222	193	193	161	165	182	186	199	207	163	175	189	201	166	166	261	261	254	254
8	BO8/05	111	137	190	210	193	193	161	161	186	190	191	203	171	171	185	197	166	174	237	261	238	254
9	BO9/05	123	123	190	222	193	193	161	165	182	182	199	207	163	175	189	201	166	166	261	261	238	254
10	BO10/05	123	125	190	226	193	193	161	161	186	186	191	207	159	163	201	201	166	166	261	261	238	254
11	BO11/05	123	125	222	226	193	193	157	161	182	186	191	207	171	171	197	201	166	174	253	261	238	254
12	BO12/05	111	123	222	226	193	193	157	161	186	190	203	203	175	175	189	201	166	166	257	261	238	242
13	BO13/05	123	125	206	214	193	193	157	161	182	190	191	207	171	175	197	197	166	174	257	261	242	242
14	BO14/05	111	123	222	226	193	193	157	165	186	186	203	207	159	159	189	201	166	166	257	261	238	242
15	BO15/05	123	123	186	214	193	193	161	165	182	186	203	207	159	171	189	201	166	174	237	261	238	242
16	BO16/05	119	137	222	222	193	193	161	165	182	186	191	207	163	175	185	201	166	174	253	261	238	242
17	BO17/05	123	123	186	222	193	193	161	161	186	190	199	199	175	175	197	201	174	174	257	261	238	242
18	BO18/05	123	125	206	210	193	193	161	165	182	186	199	199	171	175	197	201	166	166	237	237	238	242
19	BO19/05	123	127	206	222	193	193	157	161	190	190	199	203	159	171	201	201	166	174	249	261	238	250
20	BO20/05	127	133	186	222	193	193	157	161	186	186	203	203	163	163	189	189	166	166	237	257	238	254
21	BO21/05	111	123	206	206	193	193	157	161	186	190	203	203	171	175	185	197	166	166	237	261	242	242
22	BO22/05	121	123	214	214	193	193	161	165	182	182	203	203	159	163	189	197	174	174	237	253	238	254
23	BO23/05	111	125	206	206	193	193	157	161	186	190	203	203	171	175	197	201	166	174	237	253	242	250
24	BO24/05	123	123	206	222	193	193	161	165	186	186	191	203	175	175	197	201	166	166	237	261	242	254
25	BO25/05	111	137	206	218	193	193	157	161	186	190	191	203	171	175	197	201	166	166	253	261	242	250
26	BO26/05	121	123	206	222	193	193	157	161	186	190	203	207	159	163	197	197	166	174	261	261	242	246
27	BO27/05	123	125	186	214	193	193	161	161	182	190	203	203	171	175	189	197	166	174	237	257	250	254
28	BO28/05	123	125	222	222	193	193	157	161	186	190	203	203	159	175	197	197	166	166	237	237	242	250
29	BO29/05	111	137	214	222	193	193	157	161	182	186	203	203	151	175	185	197	166	166	253	261	238	242
30	BO30/05	111	137	214	222	193	193	157	165	190	190	203	203	175	175	197	197	166	166	237	253	238	242
31	BO31/05	123	123	206	206	193	193	161	165	186	190	191	191	163	175	197	197	166	166	237	261	242	242
32	BO32/05	111	123	206	210	193	193	157	165	182	186	191	203	163	175	197	201	166	174	237	237	242	250
33	BO33/05	111	137	210	222	193	193	161	165	182	186	191	191	159	159	197	201	166	166	249	253	238	254
34	BO34/05	111	123	206	222	193	193	161	161	186	186	199	199	175	175	185	201	166	166	257	261	238	238
35	BO35/05	127	133	186	222	193	193	161	161	186	186	191	191	163	175	189	189	166	166	249	261	238	238

36	BO36/05	123	123	222	222	193	193	161	161	186	186	191	207	163	175	197	201	166	174	249	257	242	242
37	BO37/05	127	133	186	206	193	193	161	165	186	186	191	191	159	175	197	201	174	174	237	237	238	238
38	BO38/05	123	127	206	210	193	193	161	165	186	186	203	203	159	175	201	201	166	174	237	261	238	242
39	BO39/05	111	123	206	214	193	193	161	161	186	190	203	203	163	175	189	197	166	166	237	261	242	250
40	BO40/05	125	125	206	222	193	193	161	161	186	186	203	203	159	171	197	201	166	166	237	261	238	242
41	BO41/05	123	125	206	214	193	193	161	161	186	190	199	203	163	171	197	197	166	166	261	261	238	250
42	BO42/05	123	125	206	214	193	193	161	161	186	190	199	203	163	171	197	197	166	166	261	261	238	250
43	BO43/05	123	125	206	206	193	193	161	161	186	186	203	207	163	171	197	201	166	174	261	261	242	250
44	BO44/05	123	125	210	214	193	193	161	161	190	190	203	203	175	175	189	189	166	174	249	261	242	242
45	BO45/05	127	137	206	206	193	193	161	161	186	190	199	203	159	175	189	197	166	166	237	257	242	254
46	BO46/05	111	125	190	222	193	193	161	161	186	186	191	203	159	171	197	197	166	166	249	261	238	250
47	BO47/05	123	127	206	222	193	193	161	161	186	186	199	199	171	175	189	197	166	166	249	261	238	254
48	BO48/05	123	127	206	206	193	193	161	161	186	186	199	199	175	175	189	189	166	166	237	249	254	254
49	BO49/05	111	127	206	210	193	193	161	161	190	190	199	203	163	175	197	201	166	174	253	261	238	254
50	BO50/05	125	127	206	222	193	193	161	161	186	190	199	203	159	171	197	201	166	174	261	261	238	250
51	BO51/05	123	123	206	222	193	193	161	161	186	186	199	203	159	175	197	197	166	174	237	261	238	238
52	BO52/05	127	137	206	214	193	193	157	161	186	186	199	203	163	175	197	201	166	174	237	253	238	242
53	BO53/05	123	125	206	222	193	193	161	165	190	190	199	203	159	175	185	197	166	166	261	261	238	242
54	BO54/05	121	137	206	222	193	193	161	161	186	190	199	203	171	171	197	201	166	166	249	257	238	238
55	BO55/05	121	125	206	214	193	193	161	161	186	190	203	207	175	175	189	201	166	174	237	261	242	242
56	BO56/05	121	125	206	214	193	193	161	161	186	190	203	207	175	175	189	201	166	174	237	261	242	242
57	BO57/05	121	123	214	222	193	193	165	165	186	186	199	203	163	175	185	197	174	174	253	253	238	242
58	BO58/05	123	125	206	210	193	193	161	161	186	190	191	191	163	175	189	201	166	166	261	261	238	242
59	BO59/05	123	123	186	206	193	193	157	161	182	190	191	199	171	175	189	201	166	166	261	261	238	242
60	BO60/05	123	125	186	222	193	193	157	161	182	190	207	207	159	163	197	201	166	166	253	261	238	238
61	BO61/05	125	127	222	230	193	193	161	161	182	186	199	203	171	171	197	201	166	166	253	261	242	254
62	BO62/05	125	127	190	206	193	193	161	161	186	190	191	191	175	175	201	201	166	166	261	261	238	250
63	BO63/05	111	127	190	206	193	193	157	165	190	190	203	203	171	171	197	197	166	174	237	261	238	238
64	BO64/05	127	127	190	206	193	193	161	161	186	186	191	199	175	175	189	197	166	166	249	257	238	254
65	BO65/05	123	137	206	206	193	193	157	161	186	190	191	199	163	171	189	189	166	174	261	261	238	242
66	BO66/05	123	123	214	222	193	193	161	165	186	190	191	207	175	175	189	189	166	174	257	261	238	238
67	BO67/05	127	127	190	226	193	193	157	161	182	186	191	203	171	179	189	197	166	174	237	237	238	254
68	BO68/05	127	137	186	206	193	193	161	161	186	190	203	203	159	163	197	201	166	166	237	261	238	238
69	BO69/05	111	121	206	214	193	193	161	161	186	186	203	203	159	163	201	201	166	166	249	261	238	238
70	BO70/05	123	123	222	226	193	193	161	165	186	186	199	199	175	179	189	201	166	174	237	261	242	242
71	BO71/05	115	133	206	210	193	193	161	165	186	190	203	203	159	175	197	201	166	174	253	261	238	254

72	BO72/05	123	127	222	222	193	193	161	161	186	190	199	203	159	163	189	201	166	174	261	261	238	238
73	BO73/05	111	125	206	206	193	193	161	161	186	190	203	203	163	175	197	201	166	166	249	261	238	242
74	BO74/05	127	133	186	222	193	193	157	161	190	190	203	203	159	171	189	201	166	166	249	261	238	238
75	BO75/06	123	123	210	222	193	193	161	161	186	186	199	199	163	175	189	201	166	166	253	261	242	254
76	BO76/06	125	127	186	222	193	193	157	161	186	190	191	207	151	175	189	197	166	166	253	261	238	242
77	BO77/06	123	123	206	206	193	193	161	161	186	190	203	203	163	175	201	201	166	174	237	261	238	238
78	BO78/06	127	133	206	222	193	193	157	165	186	186	203	203	175	175	197	197	166	166	261	261	238	254
79	BO79/07	127	137	186	214	193	193	157	161	186	186	203	207	159	163	197	201	174	174	237	261	238	242
80	BO80/07	137	137	210	222	193	193	161	161	190	190	199	203	159	175	197	201	166	174	237	249	238	242
81	BO81/07	123	127	210	222	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
82	BO82/07	123	125	186	190	193	193	161	161	190	190	203	203	163	171	189	197	166	166	237	261	238	242
83	BO83/07	127	127	210	214	193	193	157	161	182	186	203	203	171	175	185	189	166	174	261	261	238	242
84	BO84/07	127	127	210	222	193	193	161	161	182	190	203	207	171	175	185	185	166	166	261	261	238	242
85	BO85/07	125	127	186	206	193	193	161	165	186	190	203	207	159	163	189	201	166	174	261	261	238	242
86	BO86/07	127	137	206	222	193	193	161	161	182	190	203	203	159	163	189	201	166	174	249	257	242	242
87	BO87/07	111	111	206	206	193	193	157	157	186	186	199	199	171	171	201	201	174	174	253	253	238	242
88	BO88/07	121	125	222	222	193	193	161	161	186	186	203	207	163	171	189	197	166	166	237	261	238	238
89	BO89/07	115	121	206	222	193	193	161	161	186	190	203	203	171	175	197	201	166	166	257	261	238	242
90	BO90/07	121	125	210	222	193	193	161	161	190	190	199	203	163	163	189	189	166	166	261	261	242	254
91	BO91/07	123	123	214	222	193	193	165	165	186	186	199	203	163	175	185	197	174	174	253	253	238	242
92	BO92/07	123	133	206	222	193	193	161	165	182	182	203	203	171	175	201	201	166	174	237	237	238	254
93	BO93/07	119	137	222	222	193	193	161	165	182	186	191	207	163	175	185	201	166	174	253	261	238	242
94	BO94/07	125	137	206	210	193	193	161	161	190	190	199	203	159	175	197	197	166	174	237	261	238	242
95	BO95/07	123	123	214	218	193	193	157	157	182	182	199	199	163	175	185	201	166	174	237	237	238	238
96	BO96/07	123	123	186	214	193	193	161	165	182	186	203	207	159	171	189	201	166	174	237	261	238	242
97	BO97/07	121	127	206	222	193	193	157	165	186	190	191	207	171	175	197	201	174	174	237	261	238	242
98	BO98/07	111	111	190	206	193	193	161	165	182	190	203	207	171	171	185	201	166	174	261	261	238	238
99	BO99/07	123	123	190	222	193	193	157	161	186	186	207	207	163	171	189	197	166	166	257	261	238	254
100	BO100/07	115	137	186	230	193	193	157	161	186	186	203	207	159	171	201	201	166	166	261	261	238	254
101	BO101/07	125	137	186	222	193	193	157	165	186	186	207	207	163	163	189	197	166	166	261	261	254	254
102	BO102/07	123	127	222	226	193	193	161	161	186	190	203	203	163	171	189	201	166	166	261	261	238	254
103	BO103/07	115	123	218	222	193	193	157	157	186	190	199	207	163	175	197	201	166	166	253	261	238	254
104	BO104/07	123	123	226	230	193	193	161	165	186	190	203	207	171	175	201	201	166	166	253	261	238	254
105	BO105/07	115	137	186	186	193	193	161	161	186	186	191	207	163	171	201	201	174	174	261	261	238	254
106	BO106/07	123	123	222	226	193	193	157	165	186	190	207	207	171	175	189	201	166	166	261	261	238	254
107	BO107/07	123	123	206	222	193	193	161	161	186	190	191	191	163	163	197	197	166	166	253	261	242	242

108	BO108/07	123	123	206	222	193	193	157	161	182	186	191	207	175	175	197	201	166	174	237	237	242	250
109	BO109/07	123	123	214	222	193	193	157	165	186	186	191	203	159	175	201	201	166	166	261	261	242	250
110	BO110/07	123	123	206	222	193	193	157	157	186	186	203	203	175	175	197	201	166	166	237	261	242	250
111	BO111/07	111	137	214	222	193	193	157	165	190	190	203	203	175	175	197	197	166	166	237	253	238	242
112	BO112/07	123	127	186	222	193	193	157	161	186	190	191	203	159	159	189	197	166	174	237	261	242	250
113	BO113/07	123	125	186	206	193	193	157	161	186	190	199	199	163	175	185	201	166	166	261	261	238	238
114	BO114/07	123	133	186	222	193	193	161	161	186	186	191	191	175	175	185	201	166	166	249	257	238	242
115	BO115/07	127	133	186	222	193	193	161	161	186	186	191	191	163	175	189	189	166	166	249	261	238	238
116	BO116/07	123	123	214	226	193	193	161	161	186	190	191	203	163	175	185	201	166	166	253	257	242	254
117	BO117/07	111	123	186	222	193	193	157	161	182	186	191	207	163	171	197	201	166	174	253	261	238	238
118	BO118/07	123	137	210	222	193	193	157	161	186	186	191	203	159	175	185	189	166	166	261	261	238	238
119	BO01/08	111	125	206	206	193	193	157	161	190	190	203	203	171	175	197	201	166	174	237	253	242	250
120	BO02/08	121	125	186	206	193	193	161	161	182	190	203	203	163	175	197	201	166	166	249	261	242	250
121	BO03/08	121	125	186	222	193	193	161	161	190	190	203	203	159	159	201	201	166	174	237	253	242	242
122	BO04/08	111	137	206	218	193	193	157	161	186	190	191	203	171	175	197	201	166	166	253	261	242	250
123	BO05/08	123	123	206	214	193	193	161	165	182	186	191	203	159	171	197	201	166	166	261	261	242	242
124	BO06/08	123	125	206	214	193	193	161	165	182	190	203	203	171	171	197	197	166	166	261	261	242	242
125	BO07/08	123	123	206	222	193	193	161	161	186	186	199	203	159	175	197	197	166	174	237	261	238	238
126	BO08/08	123	137	206	226	193	193	161	161	186	190	199	203	171	175	189	201	166	166	237	257	242	254
127	BO09/08	111	123	190	222	193	193	161	161	182	186	191	203	171	171	197	201	166	166	257	261	238	246
128	BO10/08	123	127	206	222	193	193	157	161	186	190	191	191	171	175	189	201	166	174	261	261	238	238
129	BO11/08	111	123	186	206	193	193	161	161	182	190	199	203	171	175	185	201	166	174	261	261	238	238
130	BO12/08	123	133	186	222	193	193	161	161	186	186	191	191	175	175	185	201	166	166	249	257	238	242
131	BO13/08	123	125	186	186	193	193	161	161	186	190	191	207	163	175	185	201	166	166	249	253	238	242
132	BO14/08	127	133	222	226	193	193	161	161	186	190	199	203	171	171	185	197	166	174	253	257	238	238
133	BO15/08	123	123	214	222	193	193	161	161	186	190	191	207	171	175	197	201	166	174	237	261	238	254
134	BO16/08	127	133	214	226	193	193	161	161	186	190	203	203	163	171	189	201	166	174	237	257	238	238
135	BO17/08	123	133	206	226	193	193	157	161	190	190	191	191	175	175	189	201	166	166	237	257	238	254
136	BO18/08	115	125	206	214	193	193	161	165	182	190	191	191	163	175	189	201	174	178	237	253	238	238
137	BO19/08	123	125	206	214	193	193	165	165	182	186	199	199	163	175	189	189	166	166	237	261	242	250
138	BO20/08	123	125	206	214	193	193	157	165	186	190	199	199	163	175	189	189	174	178	237	237	242	250
139	BO21/08	123	127	210	214	193	193	161	161	186	186	203	203	163	163	189	189	166	166	237	261	238	254
140	BO01/09	123	133	206	226	193	193	157	161	182	186	199	199	171	175	189	201	166	174	249	253	242	254
141	BO02/09	125	137	206	214	193	193	157	161	186	190	199	203	163	175	189	201	166	174	261	261	238	254
142	BO03/09	123	127	206	210	193	193	161	161	186	186	199	203	159	163	189	201	166	166	261	261	254	254
143	BO04/09	123	127	206	206	193	193	161	161	182	186	203	203	163	175	185	201	166	166	261	261	242	254

144	BO05/09	127	137	206	222	193	193	161	165	182	186	203	203	171	175	189	201	166	166	257	261	238	242
145	BO06/09	123	127	206	222	193	193	161	165	182	186	199	203	171	175	189	189	166	166	249	257	238	242

APPENDIX C

Genetic profiles: Outgroup

LAB NO	TROOP	D5S1457		D10S611		D11S956		D4S243		D18S72		D1S518		D14S306		D3S1768		D15S108		D2S1326		D7S2204	
OG4	JHB Zoo	121	129	186	222	193	193	165	173	186	186	211	215	171	179	189	201	166	166	257	269	242	242
OG5	JHB Zoo	115	123	218	226	193	193	165	169	186	190	203	211	171	179	189	197	166	166	245	249	242	242
OG6	JHB Zoo	123	137	210	218	193	193	165	165	186	186	211	211	171	183	189	201	166	174	245	261	230	242
OG7	JHB Zoo	119	121	186	210	193	193	161	165	186	190	207	215	167	171	197	201	166	166	257	265	242	254
OG8	JHB Zoo	107	137	210	214	193	193	157	165	186	186	211	219	171	179	189	189	166	166	245	257	242	254

APPENDIX D

Genetic profiles: Fecal samples

LAB NO	TROOP	D5S1457		D10S611		D11S956		D4S243		D18S72		D1S518		D14S306		D3S1768		D15S508		D2S1326		D7S2204	
SBR1	Diepkloof	129	129	210	210	193	193	157	161	182	186	191	199	~	~	185	189	166	166	~	~	~	~
SBR2	Diepkloof	127	127	~	~	~	~	157	161	186	186	199	203	~	~	197	197	166	174	237	237	242	242
SBR3	Diepkloof	123	123	~	~	~	~	157	161	186	190	199	203	167	167	~	~	166	166	~	~	238	242
SBR4	Diepkloof	123	125	186	186	193	193	157	161	186	190	199	203	~	~	185	193	166	166	~	~	~	~
SBR5	Diepkloof	123	127	210	214	193	193	157	161	186	186	199	203	171	175	185	189	166	174	261	261	238	242
SBR6	Diepkloof	123	127	210	214	193	193	157	161	186	186	203	207	159	159	189	201	166	174	261	261	238	238
SBR7	Diepkloof	123	127	~	~	193	193	157	161	186	186	199	207	159	163	201	201	166	166	~	~	242	242
SBR8	Diepkloof	~	~	~	~	~	~	157	161	186	190	199	203	~	~	185	189	166	166	~	~	~	~
SBR9	Diepkloof	127	127	206	210	~	~	157	161	186	186	199	203	~	~	201	201	166	166	~	~	~	~
SBR10	Diepkloof	127	137	206	210	193	193	157	161	186	186	199	203	171	175	185	185	166	166	~	~	242	250
SBR11	Diepkloof	123	127	~	~	193	193	157	161	186	186	199	203	~	~	~	~	166	174	~	~	~	~
SBR12	Diepkloof	119	125	206	206	193	193	161	165	186	190	199	203	163	167	185	201	166	174	~	~	238	238
SBR13	Diepkloof	123	127	206	206	193	193	157	161	182	186	199	203	167	175	185	201	166	174	237	237	238	242
SBR14	Diepkloof	123	127	186	186	193	193	157	161	186	190	199	207	163	171	197	201	166	166	~	~	238	242
SBR15	Diepkloof	121	127	~	~	193	193	157	161	182	186	199	203	175	175	~	~	166	174	~	~	~	~
SBR16	Diepkloof	123	127	210	210	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
SBR17	Diepkloof	123	127	210	214	193	193	157	161	186	190	199	203	159	171	189	201	166	174	237	261	238	238
SBR18	Diepkloof	123	137	~	~	193	193	157	161	182	182	199	203	~	~	185	185	166	174	~	~	238	238
SBR19	Diepkloof	111	123	~	~	~	~	157	161	186	190	199	203	~	~	197	201	166	166	~	~	~	~
SBR20	Diepkloof	123	133	202	206	193	193	157	161	186	190	199	203	167	167	185	201	166	166	~	~	238	242
SBR21	Diepkloof	123	127	186	186	193	193	157	161	186	190	199	207	163	171	197	201	166	166	~	~	238	242
SBR22	Diepkloof	123	133	202	206	193	193	157	161	186	190	199	203	167	167	185	201	166	166	261	261	238	242
SBR23	Diepkloof	123	127	~	~	~	~	157	161	182	182	199	203	163	175	185	185	166	166	~	~	238	242
SBR24	Diepkloof	123	133	202	206	193	193	157	161	186	190	199	203	167	167	185	201	166	166	~	~	238	242
SBR25	Diepkloof	111	127	206	210	193	193	161	165	182	190	199	203	175	175	189	201	166	174	~	~	238	242
SBR26	Diepkloof	129	129	~	~	~	~	157	161	182	186	~	~	~	~	185	185	166	166	~	~	~	~
SBR27	Diepkloof	123	127	206	206	~	~	157	161	186	186	199	203	~	~	197	197	166	166	~	~	~	~
SBR28	Diepkloof	123	127	206	214	193	193	161	165	182	190	199	207	159	163	197	201	166	166	261	261	242	242
SBR29	Diepkloof	~	~	~	~	~	~	~	~	~	~	203	203	~	~	201	201	166	166	~	~	~	~
SBR30	Diepkloof	123	127	210	210	193	193	161	161	190	190	199	203	159	175	185	201	166	166	261	261	242	242
SBR31	Diepkloof	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	166	166	~	~	~	~
SBR32	Diepkloof	127	127	210	214	193	193	157	161	182	186	203	203	171	175	185	189	166	174	261	261	238	242
SBR33	Diepkloof	127	137	186	206	193	193	161	165	~	~	203	207	171	175	185	197	166	166	249	261	242	250
SBR34	Diepkloof	127	137	206	206	~	~	161	165	182	190	199	203	~	~	185	197	166	166	~	~	~	~
SBR35	Diepkloof	123	127	210	214	193	193	157	161	186	186	199	203	~	~	~	~	166	174	~	~	238	242

SBR36	Diepkloof	127	133	206	214	193	193	157	161	186	186	199	203	159	163	185	201	166	166	~	~	238	242