GENETIC DIVERSITY IN THE AFRIKANER CATTLE BREED

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GENETIC DIVERSITY IN THE AFRIKANER CATTLE BREED

(Afrikaner Cattle Breeders' Society)

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2014

DECLARATION

I declare that the dissertation hereby handed in for the qualification *Magister Scientiae* at the University of the Free State is my own work and that I have not previously submitted the same work for a qualification at/in any other University/Faculty.

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Lené Pienaar

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PREFACE

The study is represented in the form of two journal papers, augmented by two introductory chapters as well as a chapter that consists of a general discussion, conclusion and recommendations, in an effort to create a binding unit. Although care has been taken to avoid unnecessary repetition, some repetition, especially references that are necessary to explain and enlighten individual parts of the study, is unavoidable. Additional data, to bulky for publication, specifically allele frequencies of all loci by populations, is given in an Appendix.

This dissertation would not have been possible without the help of numerous people.
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LIST OF ABBREVIATIONS

Abbreviations

µL	Microliter
A	Adenine
A	Mean number of alleles
AFLPs	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
AR	Average relatedness
ARC	Agricultural Research Council
bp	Base pair
C	Cytosine
CAPN1	Calpain 1
CAST	Calpastatin
CGE	Complete generation equivalents
D_A	Nei's genetic distance
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
et al.	et alli (and others)
F	Inbreeding coefficient
f_a	Effective number of ancestors
FAO	Food and Agriculture Organization

f_e	Effective number of founders
f_g	Founder genome equivalent
f_h	Number of founder herds in reference population
Fig	Figure
F_{IS}	Population inbreeding coefficient
F_{IT}	Global inbreeding coefficient
F_{ST}	Genetic differentiation
G	Guanine
Ho	Observed heterozygosity
HWE	Hardy Weinberg Equilibrium
Hz	Unbiased heterozygosity
i.e.	id est (that is)
ISAG	International Society of Animal Genetics
K	Number of clusters
KCl	Potassium chloride
MCMC	Markov Chain Monte Carlo
$MgCl_2$	Magnesium chloride
min	Minutes
mL	Milliliter
mM	Millimolar
mtDNA	Mitochondrial DNA
N	Number of animals

N_e	Effective population size
PCR	Polymerase Chain Reaction
RAPDs	Random Amplified Polymorphic DNA
RFLPs	Restriction Fragment Length Polymorphisms
Rs	Allelic richness
SD	Standard deviation
sec	Seconds
SNPs	Single Nucleotide Polymorphisms
SSLPs	Simple Sequence Length Polymorphisms
SSRs	Simple Sequence Repeats
STRs	Short Tandem Repeats
T	Thymine
Tris-HCl	Trisaminomethane hydrochloride
U/ µL	Unit(s) per microliter
UFS	University of the Free State
UML	Unistel Medical Laboratories
v	Version
ΔF	The increase in inbreeding
ΔF_i	Individual increase in inbreeding
ΔK	DeltaK

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CHAPTER 1

THE AFRIKANER CATTLE BREED

(The Afrikaner Cattle Breeders' Society of South Africa)

1.1 Introduction

All modern domesticated cattle are descendent from a common wild ancestor known as the auroch (*Bos primigenius*); now extinct (Zeuner, 1963; Epstein & Lauder, 1971; Grigson, 1980; Epstein & Mason, 1986; Payne, 1991). However, Loftus *et al.* (1994) suggested that there were two independent domestication events of cattle, based upon mitochondrial DNA (mtDNA). Furthermore, molecular evidence provided by Loftus *et al*. (1994) suggested that *Bos indicus* has developed independently from other cattle breeds. Recent research has concluded that the current African cattle breeds originated from three different sources. Firstly, the domestication from Asia along the Nile Valley and onwards through Egypt. The second domestication event emanated through the "horn" of Africa or from the East Coast towards and through Madagascar (Payne, 1970; Epstein & Mason, 1986; Hanotte *et al*., 2002). The third theory is based on a domestication event taking place within the African continent (Grigson, 1991; Wendorf & Schild, 1994; Bradley *et al*., 1996; Hanotte *et al*., 2002).

Two main forms of domesticated cattle have already been identified with the use of molecular studies. These are European-African (*B. taurus*) and Asian (*B. indicus*) (Loftus *et al*., 1994; MacHugh *et al*., 1997). The most apparent difference between these two groups is the presence of a fatty thoracic hump in *indicus,* compared to the muscular cervico-thoracic hump in Sanga cattle (*B. taurus africanus*) (Mason & Maule, 1960). In Africa, extensive hybridization between domesticated cattle breeds have caused a complex combination of mitochondrial haplotypes indicating admixture of these two forms (Bradley *et al*., 1996). In addition, the Afrikaner, Belmont Red, Bonsmara, Nguni, Zulu, Pedi, Caprivi and Tuli cattle breeds have a submetacentric Y chromosome that cannot be distinguished from European (Taurus) breeds (Meyer, 1984; Stranzinger *et al*., 1987), but can be distinguished from Indian (Indicus) breeds (Kieffer & Cartwright, 1968).

Standards of excellence based on phenotypically observable characteristics such as colour, type, general conformation and horns have been used for centuries to compare and rate individual cattle (Heyns, 1976) and other domesticated species. Within each cattle breed, the breeders' society and the breeders themselves use phenotypic characteristics to improve the general appearance and to preserve the uniformity within the specific breed (Heyns, 1976). Conformation to standards may, however, also cause a possible loss in genetic variation.

There are a number of studies documenting the relationship between cattle breeds with the use of molecular markers (MacHugh *et al*., 1997; Blott *et al*., 1998; Luikart *et al*., 2001). However, genetic research on individual breeds is not always well documented (Berthouly *et al*., 2009; Serrano *et al*., 2009; Novoa & Usaquén, 2010; Calvo *et al*., 2011). This also applies to South Africa.

Figure 1.1 Example of an Afrikaner cow (Lené Pienaar)

Sanga cattle types are indigenous to Africa. As man continued to migrate southwards, new types of cattle breeds were developed including the Sanga and Zebu types. Breeds represented by the Sanga type are the Afrikaner (Fig 1.1), Drakensberger, Nguni (including other Nguni ecotypes such as the Pedi, Makatini, Landim and Mashona) and Tuli. By contrast, the Boran, Sokota and Masai are representative of indigenous African Zebu Types (Strydom, 2008). Meyer (1984) concluded that Sanga type cattle, with specific reference to the Afrikaner, have distinct genetic markers inherited from both *B. indicus* and *B. taurus* cattle. The species name, *B. taurus africanus*, was proposed to show that Southern African Sanga cattle such as the Afrikaner and Nguni are distinct from other African taurine cattle (Meyer, 1984; Frisch *et al*., 1997). The latter was also an indication that these breeds had mostly an ancestry of a taurine source of origin (Frisch *et al*., 1997).

Until the 1970's, the Afrikaner cattle breed was the leading and most abundant cattle breed in South Africa; and was also used for transport (Fig. 1.2) In recent years, the Afrikaner has been promoted as the ideal dam line for crossbreeding (Mostert *et al*., 1998). This breed is also present in neighbouring countries and can also be found on a global scale in countries such as Zambia, Malawi, Australia, United States etc. (Rege & Tawah, 1999).

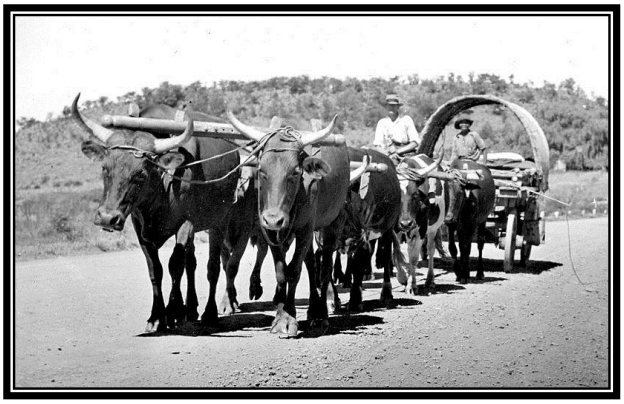

Figure 1.2 Afrikaner cattle used for transport in the early 1900's
(South African Railways & Harbours Publicity & Travel Dept)

1.1.1 Distinct characteristics of the Afrikaner breed

Cloete (1950) described Afrikaner cattle as follows: *"The Afrikaner is without doubt the king amongst all breeds of cattle. It is a masterpiece of nature and is a native of South Africa. No other life or climate can produce such a noble and elegant animal, only sunny Africa can do so".*

Characteristics such as easy calving, hardiness, outstanding carcass features and the ability to round off on natural grazing (Scholtz, 2010) are prominent attributes of Afrikaner cattle. The cows are noticeably small to medium size and have low to moderate maintenance requirements. The production of heavy weaners (when mated with large framed bulls in a crossbreeding system) is feasible. Historically, the stamina and power of the cattle were used by settlers for several different purposes (Scholtz, 2010).

In areas with well-developed agricultural sectors, such as South Africa, the management systems of populations are flexible enough to overcome extreme environmental conditions. Therefore, populations endure and exist in these harsh conditions. Consequently, small to medium bodied indigenous cows may possibly improve the output of cattle farming and succeed in these extreme conditions (Calegare *et al*., 2007).

Furthermore, the Afrikaner is known to be the breed in South Africa that carries the genetic material that contributes to a particularly tender meat – commonly referred to as the 'tenderness gene'. DNA testing for meat tenderness is a notion that has only recently been introduced. Two genes have been identified that may possibly have an effect on meat tenderness (Davis *et al*., 2008). These genes are Calpain 1 (*CAPN1*) and Calpastatin (*CAST*)

(Barendse, 2002; Page *et al*., 2002; Barendse *et al*., 2007; White *et al*., 2005). These authors concluded that the Afrikaner breed had the highest frequency, 97%, for favourable alleles at this gene marker, followed by Bonsmara (94%), Drakensberger (82%), Nguni (81%) and Tuli cattle (61%) (Banga & Van der Westhuizen, 2004). There are however several other genes that contribute to meat tenderness.

1.1.2 Crossbreeding involving Afrikaner cattle

This breed plays an important role in crossbreeding, and the development of new synthetic breeds (Scholtz, 2010). The Afrikaner breed has thus played an important role in developing six other cattle breeds, namely the Afrigus, Afrisim, Bonsmara, Hugenoot, SA Braford and Sanganer. The breed also represents a gene pool indigenous to Southern Africa with valuable attributes (Scholtz, 2010).

A problem concerning crossbreeding is the absence of measurements that can determine the genetic consequences or dilutions on the breed caused by crossbreeding (FAO, 2007). However, an important advantage of crossbreeding with indigenous breeds, such as the Afrikaner, is that the conservation and use of the cattle are being ensured, because crossbreeding needs a constant supply of purebred female animals (Scholtz & Theunissen, 2010).

1.2 The conservation of indigenous cattle breeds of South Africa

Natural and artificial selection have been important tools for the adaptation to specific (often unfavourable) environments, diseases, and improved production abilities of livestock (Panday *et al*., 2006). The conservation of unique breeds of farm animals is an essential tool for the preservation of genetic resources (Soma *et al*., 2012). It is important to realize that the objective is a long-term conservation strategy that will ensure the preservation of the widest spectrum of genetic variation for all livestock species. A FAO document (FAO, 1981) highlighted that: "*the best way of conservation would be the development of a management system which would both maintain genetic variability of existing livestock resources and at the same time permit continuous improvement in productivity and adaptability of that resource*".

The conservation and utilization of indigenous animal breeds which includes cattle, sheep, goats, pigs and chicken are currently the subject of wide interest. The reason for this is the fact that indigenous breeds may demonstrate adaptations that make them particularly suited to local environmental conditions. For example, indigenous cattle breeds within South Africa, i.e. Afrikaner, Nguni and Drakensberger, contain specific adaptive features that allows them to thrive in areas where exotic breeds cannot be produced optimally and ultimately survive, because of unfavourable conditions. Some of the adaptive features involved are heat tolerance, usage of low quality food, low water requirements for survival and resistance to diseases (Rege, 1995). Indigenous genetic resources are however under pressure from crossbreeding with exotic breeds, since unique genetic traits carried by indigenous farm animals can be lost by unrestrained crossbreeding with exotic commercial breeds. This can lead to a total loss or extinction of a breed (Ollivier & Foulley, 2002). There are major factors that can influence the risk of extinction of indigenous breeds. These include population size, frequency of crossbreeding, reproductive inefficiency, alteration in farming systems, the absence of breed societies (Chagunda & Wollny, 2003) and inbreeding depression. Conservation measures must be implemented to ensure the effective management of indigenous animal breeds (Taberlet *et al*., 2008; Boettcher *et al*., 2010).

Figure 1.3 Example of an Afrikaner bull
(The Afrikaner Cattle Breeders' Society of South Africa)

Chagunda & Wollny (2003) described the motivation of crossbreeding with exotic breeds as the intolerance for the development of successful selection systems as well as the absence of breeding objectives. Conversely, the use of indigenous domesticated breeds for crossbreeding purposes can have positive outcomes such as preserving the integrity of the genetic resources of the indigenous breed and it can contribute to the development of a new composite breed. Indigenous cattle breeds are becoming more popular in Southern Africa. Their ability to produce and reproduce under local conditions are favourable, which means that no alterations

to the environment are necessary and little to no management strategies are needed (Kars *et al*., 1994 a). Furthermore, with the use of more refined techniques such as predicted breeding values, these abilities can be further improved. However, these techniques must be used with caution, with special concern to the traits being expressed or selected for (Kars *et al*., 1994 b). There are three cattle breeds recognized as indigenous to South Africa. The Afrikaner (Fig 1.3), Nguni (Fig 1.4) and Drakensberger (Fig 1.5) cattle breeds belong to the Sanga cattle type. In addition, two other cattle breeds, namely the Bonsmara- and Tuli breeds, have also been recognized as indigenous to Southern Africa because these two breeds have been developed or bred with the use of other Sanga breeds (Scholtz, 2010).

Several characteristics within these breeds provide a great advantage over exotic breeds (Garrine *et al*., 2010; Soma *et al*., 2012). Firstly, because these breeds have been in Southern Africa for a long period of time, they were able to adapt and change to survive the environmental challenges that livestock in this region have been faced with. They are, for example, better able to withstand extreme drought conditions. The second characteristic that indigenous breeds have developed is resistance to local diseases that exotic breeds cannot tolerate or struggle to tolerate. When exotic breeds are challenged with these types of conditions, the survival rate will most probably decline more compared to that of the indigenous breeds. In addition, Hirzel (1973) suggested the food conversion rates in indigenous cattle are possibly greater than in exotic breeds under severe environmental conditions. The indigenous breeds of Southern Africa have adapted to gain the ability to withstand and perform well under different types of extreme conditions. These abilities range from tolerance to extreme high and low temperatures, high altitudes, wet or drought conditions, low quality food sources, resistance to parasites (Parfitt & Huismans, 1998) and diseases.

Figure 1.4 Example of an Nguni bull (Hannes Visagie)

Along with unique traits, the commercial value of livestock breeds is very important to secure the long term survival of a breed. This is specifically important in the indigenous cattle breeds of Southern Africa, due to the apparent lack of commercial value that these breeds have (Ramsay *et al*., 2000). Therefore, it is imperative to focus on and utilize the unique traits possessed by these indigenous breeds to make these breeds an economically sustainable and a healthy alternative to modern-day breeds (Ramsay *et al*., 2000).

Figure 1.5 Example of a Drakensberger bull
(Drakensberger Cattle Breeders' Society of South Africa)

CHAPTER 2

GENETIC DIVERSITY AND LIVESTOCK PRODUCTION

(Lené Pienaar)

2.1 Introduction to genetic diversity

Genetic diversity can be defined as the variation of alleles and genotypes present in a population, and this diversity provide a basis for adaptive and evolutionary processes (Frankham *et al*., 2002). The current pool of diversity in livestock has been created by the forces of both natural and artificial selection (Ceriotti *et al*., 2003; Groeneveld *et al*., 2010). These forces encompass processes such as mutations, adaptations, segregation, selective breeding and genetic drift (Groeneveld *et al*., 2010). Future generations of domesticated species are wholly dependent on genetic variation. Variability can be observed from genetic differences between breeds, between populations within a breed and between individuals within a population (Groeneveld *et al*., 2010). Livestock breeders' practices such as selection and animal exchanges are mostly depended on observed phenotypic diversity. In addition, ancestral diversity, natural selection and geographical isolation also play an important role in shaping current patterns of genetic diversity (Meadows *et al*., 2005).

The importance of conservation of domesticated livestock species is widely accepted; however, within-species genetic diversity is often neglected. A sub-group of a domesticated species, or better known as a livestock breed, can be defined as: "*a homogeneous, sub-specific group of domestic livestock with definable and identifiable external characters that enable it to be separated by visual appraisal from other similarly defined groups within the same species, or a homogeneous group where geographical separation from phenotypically similar groups has led to general acceptance of its separate identity*" (Turton, 1974).

There are four major themes (Chagunda & Wollny, 2003) that need to be addressed when dealing with the conservation of livestock genetic resources. These are: i) Economic – genetic variation plays an important role in providing future generations with more opportunities for selection; ii) Science - the potential expression of DNA, that is essentially the signature of adaptations and physiological functions, can be utilized scientifically for future use; iii) Culture and social - livestock forms part of the human culture and heritage and; iv) The development of animal agriculture and the sustainable utilization of livestock genetic diversity. In addition, there are other important aspects that also require attention. Firstly, knowledge concerning detailed molecular data (Weitzman, 1993; Hall & Bradley, 1995; Ruane, 2000; Bruford *et al*., 2003; Simianer, 2005; Toro & Caballero, 2005; Toro *et al*., 2009) including the within- and between breed diversity and genetic structure, is very

important. Secondly, data on population size, breed characteristics and geographical distribution are also essential for the management and conservation of a breed (Groeneveld *et al*., 2010). These features in combination with each other will provide a thorough representation of the biological variability within- and between livestock breeds.

Notter (1999) proposed that high genetic diversity in a species corresponds with favourable and diverse reproductive conditions. However, when the production conditions (or management regime) are not favourable, it will be reflected by low genetic diversity within a species. This can be in reaction to environmental pressures, the change of human nutrition requirements, disease development and other factors that continuously remain unpredictable (Chagunda & Wollny, 2003). When genetic diversity is present within a population, it is possible to select animals for a specific trait or develop a new breed.

2.2 The importance of genetic diversity

Chagunda & Wollny (2003) described the importance of genetic diversity as follows:
"Conservation of domestic animal diversity is the sum total of all operations involved in management of animal genetic resources, such that these resources are best used and developed to meet immediate and short-term requirements for food and agriculture, and to ensure the diversity they harbour remains available to meet possible long-term needs".

Two of the most important subjects when discussing the conservation of farm animal genetic resources are sustainable livestock production (de Wit *et al*., 1995) and food security (Hammond, 1994). It is of the utmost importance to maintain genetic variability within a population or a livestock breed, because without variation available animals will not be able to respond to environmental changes which forces adaptive changes to take place (Tseveenjav *et al*., 2001). When using conservation programs for conserving the optimal level of genetic variation, the main objective should be to maximize the effective population size with the use of suitable breeding systems (Gill & Hardland, 1992). This has been illustrated in previous studies done on cattle (Bodo, 1990) and milk sheep (Alderson, 1990).

The rational use of indigenous genetic resources must be supported (FAO, 2007). However, it is important to ensure that the measures taken in the current time to preserve the indigenous

livestock breeds of South Africa do not affect our ability to adjust the genetic variability of the breeds in the future.

2.3 Inbreeding and loss of genetic diversity

There are important factors that play a role in the loss of genetic variation within a breed. These factors vary from inbreeding, bottlenecks (caused by diseases, etc.), reduction in population size and decrease in range to isolation from other populations of the same species (conspecifics) (Frankham *et al*., 2002; England *et al*., 2003).

Loss of genetic variation may result in fixation at an increasing number of loci throughout the genome. Such fixation will result in an increase in homozygosity of the individuals (Lacy, 1987), with a consequent decrease in viability and ultimately inbreeding depression (Falconer, 1981; Ralls & Ballou, 1983). The loss of genetic variation within breeds will have an effect on the availability of genetic variability when demands must be met in the future, and will therefore cause difficulties (Barker, 1999).

Inbreeding is described as the mating of individuals more closely related than the average of the population and inbreeding depression is the lowering of performance and the reduction in fitness due to inbreeding, observed in the offspring from closely related individuals. The primary effects of inbreeding are the reduction of genetic variation within a population and the reduction of the performance of traits which is related to the overall fitness of an individual (Falconer & MacKay, 1996).

Inbreeding depression can be explained by two possible mechanisms. Firstly, the partial dominance theory states that when homozygosity increases in a population, deleterious- and recessive alleles becomes more prominent and visible within the population. Secondly, the over-dominance hypothesis suggests that inbreeding depression may be caused by the loss of advantageous heterozygous combinations that were present within the population (Charlesworth & Charlesworth, 1999). From these two hypotheses, there are also predictions about the future of a continuous inbreeding population. For the partial dominance theory, it is predicted that natural selection will reduce inbreeding depression through the removal of the debilitating recessive alleles. The result will be the restoration of fitness within the inbred population. In contrast with this hypothesis, the over-dominance theory states that the

continuous inbreeding will cause an increase in homozygotes, thus decreasing the amount of heterozygotes within the population and resulting in lower fitness of the whole population. In addition, the theory also predicts that no removal of debilitated recessive alleles will take place (Lande & Schemske, 1985; Barrett & Charlesworth, 1991; Roff, 2002). It has been suggested that when a population experience extreme environmental conditions, the effects of inbreeding will be more observable (Kristensen & Sørensen, 2005); thus the impact on inbreeding depression will be more profoundly expressed (Carolino & Gama, 2008).

With a large effective population size it is possible to sustain a low rate of inbreeding, within the accepted ranges of the different livestock breeds. However, a much larger effective population size is needed to preserve several alleles at a locus in a random-mating population of cattle (Denniston, 1977). Inbreeding depression impairs important traits through loss of fitness, which may impact on production characteristics (Smith *et al*., 1998). Furthermore, reproductive efficiency and survival rates are more likely to be affected by inbreeding than traits such as growth rates that have higher heritability estimates (McDaniel, 2001). When a population or breed is recovering from a reduced number of animals, the increase in genetic variability must also transpire and be available when re-establishing the population (Honda *et al*., 2007).

The short and long term consequences of inbreeding have to be considered when considering breeding programmes. In the short term, high rates of genetic improvement are usually needed. For the long term, it is important to limit the rate of inbreeding and attempt to maintain high levels of genetic variation within the breed or population (Bijma *et al*., 2001).

The reduction of levels of genetic diversity may eventually increase the susceptibility of the breed to environmental-, stochastic- and demographical variation (Shaffer, 1981; Gilpin & Soulé, 1986). In addition, a breed subjected to isolation may be more susceptible to local harsh environmental conditions and disease epidemics (Groeneveld *et al*., 2010). This may, in turn lead to increased chances of extinction (Mills & Smouse, 1994; Lacy, 1997; Frankham *et al*., 2002).

2.4 Measuring genetic diversity

DNA-based technology has several advantages, one being its suitability for the upkeep and evaluation of genetic diversity in various livestock breeds. This technology allows for the investigation of genetic variability, co-ancestry as well as the phylogenetic relationships between breeds and species (Visser & van Marle-Köster, 2011).

There are different types of molecular tools that can be used when assessing genetic diversity, with specific and unique advantages and limitations. Proteins using allozyme electrophoresis to measure genetic diversity in animal species were used during the years between 1970 and 1980. It was, however, replaced by DNA-based technology due to its inability to fully explain the extent of gene diversity (Frankham *et al*., 2010) because of the limited number of loci that can be used and the low level of polymorphism it provided (Toro *et al*., 2000). Genetic markers such as restriction fragment length polymorphisms (RFLPs), amplified polymorphic length polymorphism (AFLPs) and random amplified polymorphic DNA (RAPDs) were applied in previous years. The current markers of choice are single nucleotide polymorphisms (SNPs), mini- and microsatellites and DNA barcoding. Comparisons using these genetic markers, sequencing (either directed or large scale), mitochondrial genotyping and Y chromosomal genotyping are methods useful in measuring and characterizing genetic diversity (Rothschild, 2003) in a breed or domesticated species.

2.4.1 DNA microsatellite markers

The molecular markers chosen for the current study are microsatellites. Microsatellites are also known as short tandem repeats (STRs), simple sequence repeats (SSRs) and simple sequence length polymorphisms (SSLPs) (Bruford & Wayne, 1993). These markers have repeat motives that are highly polymorphic between breeds and even individuals, caused by a high mutation rate. The main cause of mutations within microsatellites is replication slippage. This involves the removal or addition of one or more microsatellite repeat units in the sequence during DNA replication (Schlötterer & Tautz, 1992). Mutations are a potential source of new variation within a breed (Franklin, 1982; Hill & Keightley, 1988). However, the genetic variation generated by mutations cannot be relied upon to introduce new variation in short timescales.

Microsatellites have successfully been used in previous studies as a tool to understand domestication events involving cattle, migration patterns (Bradley *et al*., 1994; Loftus *et al*., 1994; Edwards *et al*., 2000) as well as for the evaluation of genetic- variation, differentiation and the relationships within- and between cattle populations (MacHugh *et al*., 1997; Martínez *et al*., 2000; Cañón *et al*., 2001; Kim *et al*., 2002; Maudet *et al*., 2002; Panday *et al*., 2002; Dorji *et al*., 2003; Jordana *et al*., 2003). There are two different types of information that are provided by microsatellite analysis. It provides data on variations in allele frequencies between populations, as well as information on the cladistic relationships between individuals and groups (MacHugh *et al*., 1997). These DNA markers are also useful for applications such as forensic investigations, paternity testing, estimating phylogenetic relationships and determining the genetic structure of populations (Goldstein & Schlötterer, 1999).

Application of microsatellites can vary from determining the level of diversity to measuring the effects that inbreeding, population subdivision, introgression, genetic isolation and population bottlenecks have on breeds (Groeneveld *et al*., 2010). Another advantage of microsatellites is that they are hypervariable (Haymer, 1994) and has a higher mutation rate than the allozymes used before. As a result, genetic diversity can be measured on a finer scale (Hughes & Queller, 1993; Schlötterer & Pemberton, 1994). Microsatellites have the ability to show genetic admixture between populations (Machugh *et al*., 1997; Kadwell *et al*., 2001). Furthermore, they are also ideal for studying population genetic processes and are useable for genome mapping (Dayanandan *et al*., 1998). Microsatellite markers also have the capability to differentiate between individual animals when used for the assignment of breed identities from unknown samples (Diez-Tascón *et al*., 2000; Bjørnstad & Røed, 2001).

2.4.2 Quantifying genetic diversity and differentiation

The most common statistical measurements used for quantifying genetic variation are *multilocus heterozygosity*, which is the representative number of heterozygotes and the *allelic richness*, which indicates the number of different alleles presented in a population (Notter, 1999; Barker, 2001; Foulley & Ollivier, 2006). Allelic richness is a very informative measure for long-term purposes since it is more sensitive to population bottlenecks and population sizes compared to expected heterozygosity (Nei *et al*., 1975; Luikart *et al*., 1998; Leberg, 2002). Furthermore, when determining the differentiation (F_{ST}) between herds within a breed or between breeds, the genetic relationships and relatedness between them can be established.

Microsatellite-Toolkit (MSToolkit) for Excel (Park, 2001) is useful for creating input files for several statistical programs such as STRUCTURE (Prichard *et al*., 2000) and ARLEQUIN (Excoffier *et al*., 2005). In addition, this program quantifies genetic diversity within populations as unbiased heterozygosity (Hz) (Nei, 1987) and observed heterozygosity (Ho). STRUCTURE software is a widely used statistical programme to cluster individuals into groups or populations and it facilitates the determination of the genetic structure of a group of populations. The program will allow the identification of distinct genetic populations, as well as the presence of migrants and admixed individuals in a population. ARLEQUIN software is useful for quantifying genetic differentiation among populations. Another essential statistical program is DISPAN software (Ota, 1993), that measures genetic distances. With the use of pedigree information, ENDOG (Gutiérrez & Goyache, 2005) has the ability to conduct several different types of analysis, broadly defined as demographic and genetic, on pedigree data that allows for the monitoring of changes in population structure and genetic variability.

2.5 Previous genetic diversity studies in indigenous livestock breeds

A number of previous genetic diversity studies of indigenous South African livestock species, i.e. sheep, goat, chicken and cattle, are discussed briefly to illustrate how genetic studies can be used to characterise indigenous breeds.

2.5.1 Sheep

A study conducted by Peters *et al*. (2010) used blood samples from the locally developed Meatmaster sheep in South Africa for genetic analysis. This study had four specific aims: (i) to determine if a shared genetic identity can be identified within the Meatmaster sheep; (ii) to determine the genetic uniqueness of the Meatmaster compared to other sheep breeds; (iii) to determine the relationships between individual Meatmaster populations; and (iv) to determine the genetic variation within populations. The results generated in the study suggested that the Meatmaster breed have high levels of genetic diversity along with great allelic diversity. Furthermore, these allelic diversity measures will ensure the decrease in likelihood of inbreeding depression that will occur in the short term. Parentage verification and other applications can also be used in future studies with the diverse variability given by the results. Even though the Meatmaster sheep breed has only been developed recently, it shows a genetic uniqueness compare to other breeds.

The purebred Namaqua Afrikaner sheep breed indigenous to South Africa has the potential to be used in Southern African smallholder farming under harsh conditions. Therefore, Qwabe *et al*. (2013) examined the genetic variation that remained in this breeds using microsatellite markers. This author also investigated population structure. Lower levels of genetic diversity were expected due to the Namaqua Afrikaner populations involved being kept as closed populations for at least the last 15 years. Nevertheless, the genetic diversity values reported in this study were comparable to values reported for other indigenous sheep breeds. Furthermore, significant differentiation between the three populations was suggested by the population structure results. Follow-up studies are also suggested and it was recommended that these should be performed every five years, to ensure minimal inbreeding and maintenance of the current level of genetic diversity.

Soma *et al*. (2012) conducted a genetic analysis of 20 different sheep breeds found in South Africa. These included are indigenous South African breeds namely the Namaqua Afrikaner, Ronderib Afrikaner, Pedi, Swazi and Zulu. The aims of the study were to evaluate the genetic relationships between the sheep breeds and measure the correlation between the patterns of differentiation as well as the levels of genetic diversity to known breed histories. Microsatellite markers were used to determine genetic diversity and differentiation (or alternatively similarities) between the breeds. High levels of heterozygosity were observed in most of the indigenous breeds, but with comparatively low values in the fat-tailed breeds. Furthermore, population structure results showed a distinction between the fat-tailed indigenous breeds and both the North African/Middle Eastern breeds and the breeds of European origin. The authors concluded that, compared to the other indigenous breeds studied, the Damara breed has the most secure future because it is farmed commercially by a large number of farmers in South Africa and Namibia. Breeds that are farmed in relatively low numbers and are considered as threatened are the Afrikaner, Pedi and Zulu. Continued genetic monitoring of the latter breeds is therefore crucial.

2.5.2 Goat

The Kalahari Red goat breed was genetically analysed by Kotze *et al*. (2004). The aims of the study were: (i) to optimize specific microsatellite markers for goats; (ii) to determine the genetic diversity of the markers within Kalahari Red goat populations; and (iii) to use

molecular inventories to describe the Kalahari Red goat breed. It was concluded that the primers used in the study met all the criteria and can therefore be used and will contribute to the standardization of future studies involving the genetic characterization of Kalahari Red goat populations. Application such as parentage verification and forensic analysis will also be possible. Information including the characterization, a DNA repository and detailed genetic diversity has been established for this goat breed and will be used in future management and development strategies.

The Tankwa goat breed is indigenous to South Africa. Animals of this type have been free roaming in the Tankwa Karoo National Park for more than 50 years, with a population size varying between 100 and 300, and differentiation from other breeds reportedly developed quickly. Kotze *et al*. (2014) aimed to determine the possible uniqueness of the Tankwa goat breed. This was the first attempt to gain genetic information on this indigenous South African goat breed. Population structure analysis based on Bayesian- and frequency based statistical methods suggested uniqueness in the breed. Drift that is a result from decades of isolation and an adaptation component caused by natural selection may have caused this uniqueness the Tankwa breed. The results also suggested high levels of genetic variability.

2.5.3 Chicken

Mtileni *et al*. (2010) conducted a study on South African field and conserved chicken populations. This study was undertaken to study the maintenance and preservation of within-species genetic diversity of chickens. Reference populations from other African countries such as Malawi, Sudan and Zimbabwe were also used.

The results from the study suggested that there is more genetic variability in field populations than compared to conservation chicken populations. The genetic structure results also showed that genetically distinct populations could be identified between conserved and field populations. High heterozygosity deficiency levels were observed in the conservation flocks compared to the field chicken populations. Results from the inbreeding coefficient (F_{IS}) demonstrated that the conservation flocks have significant genetic diversity and low levels of inbreeding.

2.5.4 Cattle

No genetic diversity studies have been conducted on indigenous South African cattle breeds. Therefore, a study on Colombian Brahman cattle is discussed below as an example of work on a localized cattle breed. This study by Novoa & Usaquén (2010) aimed to determine the level of genetic diversity of the Brahman cattle (*B. indicus*) breed in Colombia. This breed has been under constant selection pressures and subjected to inbreeding and genetic drift. In addition, migration of Brahman cattle from both farms and other countries has also been a continuous practice. The aims of the study were to determine the genetic relationship with other zebu and taurine breeds.

It was concluded that only a single genetic population of the Brahman cattle exists in Colombia. Reliable paternity testing can be conducted due to the lack of population substructures. Furthermore, the high levels of genetic diversity and unique alleles are present within the population will create a reservoir with potentially exclusive alleles and genetic material.

The preceding sections clearly demonstrate the importance of genetic diversity studies on indigenous livestock breeds, including cattle. However, this review also highlights the lack of such studies involving indigenous cattle breeds, which motivated the current study.

2.6 Aims of this study

Three hypotheses to be tested were formulated for the current study: Firstly, low levels of genetic diversity will be observed in the Afrikaner breed; secondly, it is assumed that a significant component of the overall diversity resides in commercial as opposed to stud herds; and thirdly, the current level of inbreeding present in this cattle breed is higher compared to reported values for local breeds.

Studies such as this one, can contribute to the preservation of indigenous livestock, and are both important and necessarily in South Africa. The conservation of the Afrikaner cattle populations as a whole and the maintenance of acceptable levels of genetic diversity within individual herds, will ensure that this indigenous cattle breed in South Africa perform optimally. From a long-term conservation point of view, the genetic resources in indigenous livestock animals must be secured, since an unknown future in terms of environmental

conditions may cause future threats to livestock production (Taberlet *et al*., 2011; Scholtz *et al*., 3013).

To describe the existing level of genetic variability of the Afrikaner cattle breed and thus address these hypotheses, the state of genetic variability and the rate of inbreeding in these cattle populations must be determined. The specific aims of the study were therefore:

1. To compare the level of genetic variation between herds, therefore identifying the remaining resources of heterozygosity within the breed.
2. To determine the component of diversity that still resides in commercial as opposed to stud herds.
3. To compare the genetic variability estimations obtained from a pedigree analysis study on the Afrikaner breed with the levels of inbreeding estimated using microsatellite- and pedigree.

CHAPTER 3

GENETIC DIVERSITY IN SELECTED STUD AND COMMERCIAL HERDS OF THE AFRIKANER CATTLE BREED

(Lené Pienaar)

3.1 Abstract

The Afrikaner is one of three indigenous cattle breeds found in South Africa. Until the 1970's the Afrikaner was the most abundant indigenous breed in the country. The Afrikaner was also extensively used for crossbreeding purposes and breed development. Six other composite breeds were developed from crosses with the Afrikaner. To assess the level of genetic diversity in the breed, a genetic evaluation was initiated. The objective of this study was to determine the genetic diversity of selected stud and commercial herds from the whole Afrikaner population, as well as determine the genetic structure among these herds. A total of 1214 stud animals (representing 28 herds) and 166 commercial animals (nine herds) from different geographical areas in South Africa were included in this study. Animals were genotyped at the two major animal molecular laboratories in South Africa, with both using the same 11 microsatellite marker set. Assignment methods (based on STRUCTURE software) revealed a real structure consisting of four genetic populations (K=4). Estimates of genetic diversity did not support the hypothesis of significant loss of genetic diversity in any individual Afrikaner herd. Heterozygosity estimates ranged from 0.456-0.737 within individual populations, with an overall heterozygosity estimate of 0.568 for the Afrikaner breed; and the average number of alleles per locus was 2.67-7.78, with an average of 5.18 alleles per locus. A total of 424 from 703 pair-wise combinations between herds supported the hypothesis of significant differentiation. However, no consistent pattern of significant differentiation between stud- and commercial herds could be identified. It is concluded that a moderate to high degree of variation is still present within the Afrikaner cattle breed, despite the recent decline in numbers of this indigenous breed.

3.2 Introduction

The Afrikaner cattle breed (*Bos taurus africanus*) is an indigenous South African breed of the "Sanga" type. Sanga cattle are generally found in Southern Africa and are an admixture of *Bos indicus* and *Bos taurus* breeds (Payne & Wilson, 1999). Sanga cattle therefore contain genetic material that has been inherited from both cattle species (Meyer, 1984). The Afrikaner breed is well adapted to all local cattle producing areas and can be found in various geographical areas in and around Southern Africa. In addition, this breed has extensively been used for crossbreeding purposes and for breed development. Six other composite breeds were developed from the Afrikaner. This could have been one of the reasons for the

significant decline in the number of pure Afrikaner animals. Until the 1970's, the Afrikaner was the most abundant indigenous cattle breed in South Africa. However, problems encountered by farmers, such as perceived high levels of inbreeding, lowered fertility and a decreased reproductive period (Coetzer & Van Marle, 1972) in cows subsequently caused a significant decline in the popularity and numbers of this breed.

Genetic diversity is required for populations to provide for adaptation to different environmental pressures and can be defined as the variation of alleles and genotypes presented in a breed. This provided a basis for adaptive and evolutionary processes (Frankham *et al*., 2002). The current level of diversity in livestock has been created by the combined forces of both natural and artificial selection. These forces can be described as mutations, adaptations, segregation, selective breeding and genetic drift (Groeneveld *et al*., 2010). Genetic diversity in livestock is essential for the adaptive responses needed in ever-changing farming conditions (FAO, 1998; Bennewitz *et al*., 2006) and ultimately to respond to the challenges created by climate change. Diversity also provides a reservoir for genetic variation to ensure that future market demands can be met through selection (FAO, 1998). Knowledge of genetic variation measured by DNA markers within- and between herds can be used to: (i) characterize breeds (Hetzel & Drinkwater, 1992) and thus conserve farm animal genetic resources for different social-, historical-, cultural- (Gandini & Villa, 2003) and economic (Simianer *et al*., 2003) reasons; (ii) for the genetic improvement of breeds for production and conservation purposes (Dadi *et al*., 2008); and (iii) delineate demographic factors which play a major role in the conservation and characterization of unique livestock breeds (Malevièiuto *et al*., 2002).

Little is known about the generic variation that still resides within the Afrikaner breed and it is therefore important to evaluate the level of genetic variation in this breed. It is important to restrict inbreeding to acceptable levels in breeds and individual herds to avoid deleterious effects on fitness traits, thereby, ensuring viability (Fernández *et al*., 2005). Data on diversity in the Afrikaner could thus be used to determine what measures should be taken to ensure the survival of future generations of this indigenous breed.

Microsatellite markers are ideal for evaluating the genetic diversity within- and between breeds (Barker, 1999). These markers have repeats motives that are usually highly polymorphic between breeds and even individuals. Microsatellites have successfully been

used in previous studies as a tool to understand domestication events involving cattle, migration patterns (Bradley *et al*., 1994; Loftus *et al*., 1994; Edwards *et al*., 2000) as well as for the evaluation of genetic- variation, differentiation, relationships within- and between cattle populations and patterns of migration of African cattle (MacHugh *et al*., 1997; Martínez *et al*., 2000; Cañón *et al*., 2001; Rege *et al*., 2001; Hanotte *et al*., 2002; Kim *et al*., 2002; Maudet *et al*., 2002; Panday *et al*., 2002; Dorji *et al*., 2003; Jordana *et al*., 2003; Sun *et al*., 2008; Novoa & Usaquén, 2010; Sharma *et al*., 2013). It should be kept in mind that the markers used for genotyping cattle in South Africa have specifically been designed for European cattle breeds. Problems have been reported where parentage verification could not be established due to some Afrikaner individuals being homozygous at a large number of loci (Marx, personal communication). Therefore, it may be possible that the results generated by these standardized markers, may not be wholly appropriate for indigenous breeds.

The aims of the current study were: (i) to determine the level of genetic diversity within pure Afrikaner cattle stud- and commercial herds, and thus identify the remaining reservoirs of heterozygosity within the breed; (ii) to determine the genetic structure of the breed and elucidate patterns of differentiation between herds; and (iii) to screen for genetic differences between stud and commercial herds.

Differences between stud- and commercial Afrikaner herds can be ascribed to differences in breeding objectives. Whereas commercial breeders tend to focus on economic traits such as reproduction and production, stud breeders tend to also pay attention to breed standards, which are in many cases artificial standards that are not linked to production (Scholtz, 2005).

3.3 Materials and methods

3.3.1 Sample collection

Genotypes for stud animals were generated by the Animal Improvement Institute at the Agricultural Research Council (ARC) and at the Unistel Medical Laboratories (UML). Samples originated from different geographical areas within South Africa, particularly in the Free State, Northwest, Limpopo Provinces and as far afield as Namibia (Fig. 3.1). Altogether 1214 pure stud animals from 28 herds were genotyped. The stud samples used in the current study were specifically used for parentage verification; therefore all animals within a given herd were most likely to a degree related.

Both Laboratories used the same standardized molecular markers to generate genotypes for the cattle, as recommended by the International Society of Animal Genetics (ISAG). This set consists of 11 polymorphic dinucleotide microsatellite markers to be used for parentage testing on cattle as well as tests for genetic diversity (http://www.projects.roslin.ac.uk). The markers are listed in Table 3.1.

In addition, a total of 190 samples were collected from pure commercial Afrikaner animals from nine different geographic areas in South Africa (Fig. 3.1). It was attempted to use unrelated animals for genotyping. In cases where it was impractical to identify the relationship between animals due to a lack of pedigree information, individual cattle was randomly chosen from the herd. Plucked hair of the tail from each individual animal was used for DNA analysis. Non-invasive methods for obtaining biological samples are becoming increasingly popular due to advantages relating to cost, time and logistics while still providing ample DNA for molecular studies (Taberlet & Bouvet, 1992; Morin & Woodruff, 1992; Taberlet *et al*., 1997). Hair samples were stored in a cool dry area with individual samples in separate sealed envelopes**.**

Table 3.1 Loci used, chromosomal location, primer sequences (forward and reverse) of eleven primer pairs, detected size ranges and references

Locus	Chromosome	Primer Sequences	Size Range (bp)	References
BM1824	D1S34	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACTGCTTCCTTG	170 - 218	Barendse *et al*. (1994)
BM2113	D2S26	F: GCTGCCTTCTACCAAATACCC R: CTTCCTGAGAGAAGCAACACC	116 - 146	Sunden *et al*. (1993)
ETH10	D5S3	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC	198 -234	Solinas-Toldo *et al*. (1993)
INRA23	D3S10	F: GAGTAGAGCTACAAGATAAACTTC R: TAACTACAGGGTGTTAGATGAACTC	193 - 235	Vaiman *et al*. (1994)
SPS115	D15	F: AAAGTGACACAACAGCTTCACCAG R: AACCGAGTGTCCTAGTTTGGCTGTG	235 - 265	Baylor College of Medicine Human Genome Sequencing Center (2006)
TGLA53	D16S3	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA	147 - 197	Georges & Massey (1992)
TGLA122	D21S6	F: AATCACATGGCAAATAAGTACATAC R: CCCTCCTCCAGGTAAATCAGC	134 - 193	Georges & Massey (1992)
TGLA126	D20S1	F: CTAATTTAGAATGAGAGAGGCTTCT R: TTGGTCCTCTATTCTCTGAATATTCC	104 - 131	Georges & Massey (1992)
TGLA227	S18S1	F: GGAATTCCAAATCTGTTAATTTGCT R: ACAGACAGAAACTCAATGAAAGCA	64 - 115	Georges & Massey (1992)
ETH3	D19S2	F: GAACCTGCCTCTCCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	90 - 135	Solinas-Toldo *et al*. (1993)
ETH225	D9S2	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	136 - 165	Steffen *et al*. (1993)

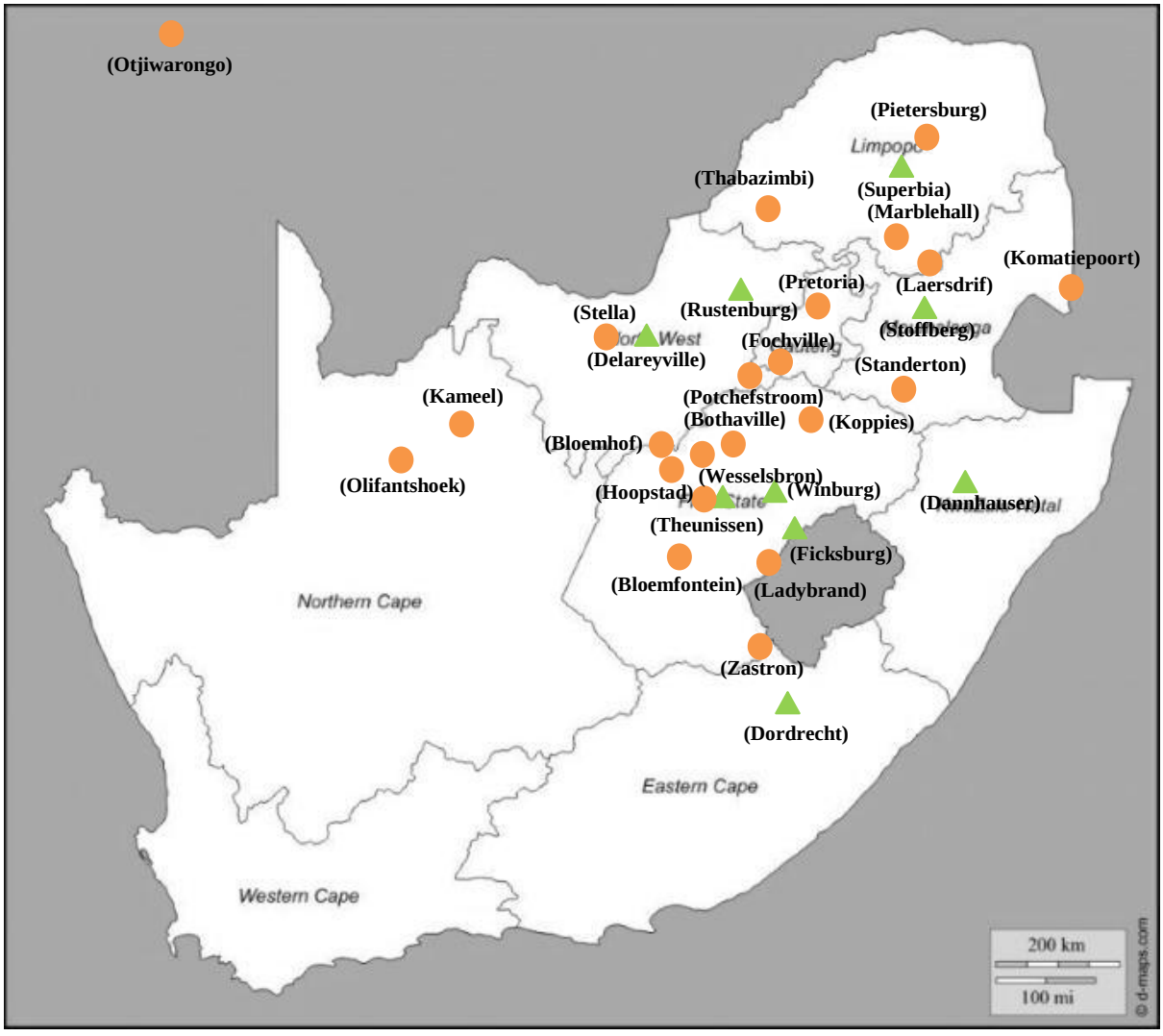

Figure 3.1 Geographical distributions of Afrikaner stud- (●) and commercial (▲) herds in South Africa and Namibia selected for genetic analysis

3.3.2 Molecular techniques

A direct Polymerase Chain Reaction (PCR) technique was used during this project. A total of three to five hairs per animal were washed with distilled water and air dried for ten minutes. Hair follicles for each individual hair were then cut into 0.2 mL PCR tubes. PCR mixtures contained 1.0 µL of primer mix (11 microsatellites), 3.67 µL deionized water, 0.18 µL Tween, 0.75 µL dNTP's, 1.50 µL Supertherm Gold reaction buffer (20 mM Tris-HCl pH 8.3, 15 mM MgCl2, 50 mM KCl), and 0.4 µL Supertherm Gold DNA polymerase (5 U/ µL) (total volume 7.5 µL), and 3-4 hair follicles. Reaction conditions consisted of a 10 min Hot Start® polymerase activation step at 95 °C, followed by 33 cycles of 45 sec denaturation at 94 °C, 90 sec annealing at 61 °C, 60 sec extension at 72 °C; and a final extension step at 72 °C for 60 min. The Genetic Analyzer 3130xl by Life Technologies was used for fragment analysis. Results were screened using GeneMapper® Software, version 4.0.

3.3.3 Statistical analysis

Genetic diversity within herds, expressed as unbiased heterozygosity (Hz) (Nei, 1987) and the mean number of alleles (A) per locus was calculated with the use of Microsatellite-Toolkit (MSToolkit) for Excel (Park, 2001). In addition, MSToolkit is useful for creating input files for several statistical programs such as ARLEQUIN (Excoffier *et al*., 2005), FSTAT (Goudet, 2002), STRUCTURE (Pritchard *et al*., 2000) and DISPAN (Ota, 1993). Allelic richness (Rs) for each herd was determined as an additional measure of diversity that compensate for unequal sample sizes, using FSTAT 2.9.3 software. Furthermore, FSTAT was also used to calculate unbiased F-statistics (Wright, 1951; Weir & Cockerham, 1984), as the mean within population inbreeding coefficient or F_{IS}, which measures possible heterozygote deficiency; and the global inbreeding coefficient or F_{IT}. ARLEQUIN software was used to screen for deviations from expected Hardy Weinberg equilibrium (HWE).

To describe the genetic structure, STRUCTURE software was used to implement a Bayesian-based assignment approach for multilocus genotype analysis. Individuals were assigned to clusters (K) and admixture proportions of individuals were estimated for the combined stud and commercial database. All runs consisted of a burn-in period of 100 000 steps that were followed by 200 000 Markov Chain Monte Carlo (MCMC) iterations. Structure Harvester v0.6.93 (Earl & vonHoldt, 2012) was used to determine DeltaK (ΔK) (Evanno *et al*., 2005)

from -Ln probability values, to determine the correct number of clusters identified with STRUCTURE software.

Genetic differentiation was also inferred from F_{ST} values, using ARLEQUIN software. Significance of deviations from the hypothesis of zero differentiation at a level of $p > 0.05$ were determined. The Bonferroni correction method (Bland & Altman, 1995) was applied to compensate for multiple pair-wise comparisons. Nei's genetic distance (D_A, Nei *et al*., 1983) among herds was estimated using Dispan software (Ota, 1993). The Dispan software was also used to create a tree from D_A values, which was viewed using TreeView (Page, 1996).

3.4 Results

Allelic polymorphism was observed at all loci studied. The number of alleles per locus ranged from eight alleles at locus *BM1824* to 19 at locus *TGLA53*. Due to difficulties in genotyping, the loci *ETH3* and *ETH 225* were excluded from further analysis. Consequently, only nine microsatellite loci, *BM1824*, *BM2113*, *ETH10*, *INRA23*, *SPS115*, *TGLA53*, *TGLA122*, *TGLA126* and *TGLA227* were used in the remaining statistical analysis.

The unbiased heterozygosity (Table 3.2) ranged from a low of 0.456 ± 0.085 in the Pietersburg (PI) herd to a high of 0.737 ± 0.043 in the Fochville1 (FO1) herd. Out of the 37 herds studied, only two herds - Kameel1 (KA1) and PI - showed heterozygosity values below 0.5, with values of 0.489 ± 0.066 and 0.456 ± 0.085 respectively. The overall Hz average of the breed across herds was 0.568 ± 0.067 and with an average of 5.18 ± 1.76 alleles per locus. Within individual populations, the mean number of alleles (A) per locus ranged from 2.67 to 7.78. The Potchefstroom herd (PO) had on average the greatest mean number of alleles per locus and Bothaville1 (BO1) the lowest mean number of alleles. Results correlated with known herd history. For example, the Potchefstroom herd is a mixture of different genotypes that were acquired from several farmers in the North West district, thus the large number of alleles was expected. There was a clear and positive correlation between sample size and the mean number of alleles observed. In particular, herds with a sample size smaller than 10 consistently possessed a mean number of alleles value below 4.0. Allelic richness (Rs) estimates were therefore used for a more accurate view of levels of diversity. The FO1 herd possessed the highest Rs value of 3.50 ± 0.623, whereas BO1 showed the lowest Rs value at 2.139 ± 0.549. Furthermore, it should also be noted that only three herds, namely FO1, FO2

and Bothaville2 (BO2), had Rs estimates higher than three. This is consistent with the heterozygosity estimates where these three herds were also the herds with the highest Hz values.

Estimates of F_{IT} and F_{IS} were 0.017 ± 0.005 and -0.024 ± 0.005 respectively. Therefore, the total inbreeding coefficient was 1.7 %. It was assumed that F_{IS} values of -1 were indicative of an excess of heterozygotes presumably indicating outbred populations, whereas values of 1 suggest heterozygote deficiency (Paiva *et al*., 2011). Pretoria (PR) were the herd with the highest F_{IS} estimate with 0.107; therefore the herd with the highest average of homozygous individuals (10.7 %). The Zastron (ZA) and Ficksburg (FI) herds also had estimates higher than 0.5, with 0.064 (6.4 %) and 0.051 (5.1 %) respectively. Bothaville2 (BO2), Standerton (ST) and Fochville2 (FO2) showed no significant F_{IS} values. All other herds showed estimates below -0.010. Herds with an estimate lower than -0.100 were Marblehall (MA), Wesselsbron (WE), Bothaville1 (BO1), Stella (STE), Ladybrand (LA), Hoopstad (HO) and Kameel2 (KA2).

Overall, few individual **herds** deviated significantly from the expected HWE (Table 3.3). Eight out of nine **loci** tested did, however, deviated significantly from expected HWE in one or more populations. The exception was locus *ETH10* which showed no deviation from HWE in any population. A further two loci (*BM2113* and *TGLA227*) showed deviations from HWE for only one herd each, Komatiepoort (KO) and Pretoria (PR). A total of 26 herds showed no deviations from expected HWE. Other herds showed significant deviations at 1 to 2 loci, with only two herds, BO2 and Thabazimbi (THA), showing deviations at three loci. In general, it was observed that the commercial herds deviated less from Hardy-Weinberg equilibrium compared to the stud herds. This was expected because of the different selection intensities and migration patterns in stud- and commercial herds.

Table 3.2 Genetic diversity results of Afrikaner stud and commercial cattle herds based on nine microsatellite markers. The parameters are: unbiased heterozygosity (Hz), herd name abbreviation, herd sample size (N), mean number of alleles (A) and allelic richness (Rs). The abbreviation SD denotes standard deviation. Values in colours are **highest** and **lowest** for each parameter

Herd	Abbr.	N	Hz ± SD	A ± SD	Rs ± SD	F_{IS}
Marblehall	MA	15	0.525 ± 0.075	3.44 ± 1.24	2.430 ± 0.688	-0.105
Bothaville	BO1	6	0.507 ± 0.067	**2.67 ± 0.71**	**2.139 ± 0.549**	-0.316
Kameel1	KA1	17	0.489 ± 0.066	3.89 ± 1.05	2.403 ± 0.599	0.026
Pietersburg	PI	7	**0.456 ± 0.085**	3.00 ± 1.12	2.319 ± 0.784	-0.035
Bothaville	BO2	20	0.666 ± 0.039	5.22 ± 1.79	3.007 ± 0.497	-0.005
Olifantshoek	OL	17	0.562 ± 0.073	4.11 ± 1.76	2.622 ± 0.679	-0.096
Wesselsbron	WE	6	0.532 ± 0.105	3.44 ± 1.67	2.644 ± 1.061	-0.166
Stella	STE	6	0.504 ± 0.061	2.78 ± 0.67	2.344 ± 0.562	-0.199
Thabazimbi	THA	29	0.601 ± 0.077	5.33 ± 2.35	2.819 ± 0.751	-0.061
Standerton	STA	18	0.589 ± 0.073	4.11 ± 1.54	2.699 ± 0.739	-0.006
Otjiwarongo	OT	14	0.627 ± 0.074	5.44 ± 2.30	2.999 ± 0.844	-0.065
Zastron	ZA	14	0.550 ± 0.068	3.89 ± 1.27	2.589 ± 0.700	0.064
Ladybrand	LA	10	0.595 ± 0.080	4.33 ± 1.80	2.780 ± 0.803	-0.127
Hoopstad	HO	6	0.554 ± 0.081	3.33 ± 1.22	2.616 ± 0.808	-0.115
Theunissen1	TH1	16	0.522 ± 0.072	3.44 ± 1.42	2.380 ± 0.634	-0.039
Theunissen2	TH2	8	0.600 ± 0.081	3.67 ± 1.66	2.720 ± 0.863	-0.018
Theunissen3	TH3	13	0.540 ± 0.071	3.44 ± 1.13	2.473 ± 0.614	-0.021
Theunissen4	TH4	124	0.594 ± 0.078	5.56 ± 2.46	2.784 ± 0.768	-0.024
Komatiepoort	KO	48	0.569 ± 0.073	5.44 ± 2.60	2.668 ± 0.703	0.029
Potchefstroom	PO	236	0.552 ± 0.063	**7.78 ± 2.11**	2.609 ± 0.609	-0.013
Kameel2	KA2	6	0.526 ± 0.067	3.22 ± 0.97	2.486 ± 0.607	-0.110
Bloemfontein	BL	163	0.615 ± 0.074	6.44 ± 2.24	2.872 ± 0.720	0.004
Pretoria	PR	7	0.529 ± 0.085	3.44 ± 1.51	2.589 ± 0.913	0.107
Fochville1	FO1	23	**0.737 ± 0.043**	6.89 ± 1.69	**3.503 ± 0.623**	-0.043
Fochville2	FO2	35	0.663 ± 0.044	6.67 ± 2.12	3.116 ± 0.558	-0.001
Koppies	KOP	14	0.501 ± 0.082	3.44 ± 1.33	2.397 ± 0.771	-0.047
Laersdrif	LAE	69	0.593 ± 0.062	6.11 ± 2.09	2.784 ± 0.624	-0.088
Bloemhof	BLO	267	0.570 ± 0.073	7.44 ± 2.13	2.723 ± 0.722	-0.030
Theunissen	TH	18	0.640 ± 0.055	5.56 ± 1.67	2.979 ± 0.689	0.027
Dannhauser	DA	14	0.556 ± 0.061	5.11 ± 1.83	2.713 ± 0.711	-0.030
Dordrecht	DO	20	0.560 ± 0.047	4.56 ± 1.13	2.583 ± 0.437	-0.095
Ficksburg	FI	20	0.579 ± 0.078	4.44 ± 1.81	2.736 ± 0.785	0.051
Stoffberg	ST	19	0.591 ± 0.073	5.22 ± 1.86	2.820 ± 0.777	-0.019
Rustenburg	RU	18	0.580 ± 0.072	4.33 ± 1.50	2.686 ± 0.781	0.010
Superbia	SU	17	0.525 ± 0.069	3.33 ± 1.41	2.387 ± 0.613	-0.099
Delareyville	DE	20	0.512 ± 0.072	5.22 ± 2.33	2.484 ± 0.756	-0.074
Winburg	WI	20	0.600 ± 0.047	4.67 ± 1.58	2.837 ± 0.882	0.010

Table 3.3 Conformation to expected Hardy-Weinberg Equilibrium (HWE) for nine loci and 28 Afrikaner stud- and commercial herds. When p > 0.05 loci did not deviate from HWE. Loci at a herd that deviated from HWE where p < 0.05 are presented with an asterisk (*)

Herd	BM1824	BM2113	ETH10	INRA23	SPS115	TGLA53	TGLA122	TGLA126	TGLA227	TOTAL
MA	0.599	0.523	1.000	0.612	MONO	0.678	0.714	0.376	0.199	0
BO1	0.636	1.000	0.638	1.000	MONO	1.000	1.000	0.637	0.395	0
KA1	0.350	0.276	0.846	0.820	1.000	0.164	0.759	1.000	0.362	0
PI	1.000	1.000	0.091	1.000	MONO	1.000	1.000	0.105	0.639	0
BO2	0.968	0.133	0.296	0.288	*0.013	0.820	*0.038	*0.026	0.951	3
OL	0.612	0.720	0.338	0.169	MONO	1.000	0.532	0.811	0.458	0
WE	0.638	0.916	1.000	0.668	MONO	0.139	0.515	MONO	0.209	0
ST	1.000	1.000	1.000	0.516	1.000	0.273	0.394	1.000	0.395	0
THA	*0.039	0.845	0.394	*0.032	MONO	*0.018	0.541	0.906	0.947	3
STA	1.000	0.404	0.217	1.000	1.000	1.000	0.526	0.665	0.686	0
OT	0.647	0.579	0.404	0.666	1.000	0.082	0.276	0.582	0.666	0
ZA	1.000	0.890	1.000	0.182	1.000	0.233	0.277	0.062	0.741	0
LA	0.068	0.901	0.615	0.106	MONO	1.000	1.000	0.242	0.967	0
HO	1.000	0.633	1.000	1.000	MONO	0.509	0.757	1.000	0.204	0
TH1	1.000	0.861	0.273	1.000	MONO	0.105	0.106	0.925	0.160	0
TH2	0.441	0.863	1.000	0.782	MONO	0.393	0.612	0.186	0.855	0
TH3	0.209	0.561	0.167	0.900	MONO	0.456	0.200	0.684	0.821	0
TH4	0.727	0.613	0.486	0.116	MONO	0.922	0.599	0.877	0.515	0
KO	0.804	*0.025	0.250	0.139	MONO	0.345	0.338	*0.012	0.892	2
PO	0.368	0.658	0.445	0.249	*0.006	0.731	0.778	0.856	0.560	1
KA2	0.248	0.756	1.000	0.793	1.000	1.000	0.515	1.000	1.000	0
BL	*0.003	0.490	0.180	0.867	1.000	0.524	0.491	0.927	0.112	1
PR	0.509	0.978	0.663	1.000	MONO	1.000	1.000	0.851	*0.007	1
FO1	0.635	0.783	0.933	*0.008	0.434	0.186	0.775	0.475	0.279	1
FO2	0.297	0.475	0.908	0.819	0.076	0.227	0.798	0.645	0.446	0
KOP	0.046	0.849	1.000	*0.004	MONO	*0.033	0.173	*0.038	0.897	3
LAE	0.750	0.563	0.643	0.186	0.191	0.926	0.056	0.407	0.692	0
BLO	0.571	0.551	0.988	0.394	1.000	0.379	0.983	0.244	0.815	0
TH	0.840	0.282	0.525	0.441	1.000	0.455	0.175	*0.036	0.380	1
DA	0.741	0.176	0.847	1.000	1.000	0.369	0.207	0.529	0.690	0
DO	0.369	0.727	0.344	0.738	1.000	0.319	0.824	0.702	0.833	0
FI	0.715	0.529	0.587	0.227	MONO	0.880	0.196	0.316	0.592	0
ST	0.063	0.953	0.207	0.479	1.000	0.965	0.591	0.150	0.650	0
RU	0.873	0.862	0.200	0.072	1.000	0.673	*0.031	0.152	0.435	1
SU	1.000	0.157	0.244	0.820	MONO	0.403	0.609	0.719	0.298	0
DE	1.000	0.383	0.110	0.221	1.000	*0.010	0.885	1.000	0.260	1
WI	0.789	0.702	0.727	0.175	1.000	0.815	0.681	0.199	0.468	0

The Bayesian assignment approach using STRUCTURE and associated Structure-Harvester software showed that the samples from 37 herds had the highest probability of representing only four genetic clusters, with K=4 (from DeltaK values). The geographical distribution of the stud and commercial herds were not a contributing factor to the assignment of populations to specific clusters. Furthermore, the identity of populations as stud or commercial herds (as presented in Table 3.4) could also not be confirmed from Bayesian analysis, therefore no clear differences between these two types of herds could be observed from genetic structure. With a criterion of 50%, i.e. where at least 50% of the members of a populations grouped to a single cluster, membership of the four identified clusters were populated as follows: Cluster 1 contained herds from Bothaville2, Wesselsbron, Otjiwarongo, Ladybrand and Focville1 (with 50% or more of each herd resorting to the cluster); Cluster 2 contained Hoopstad, Theunissen1 and Theunissen4; Cluster 3 with Bloemhof; and Cluster 4 with 50% or more animals from each of the Pietersburg and Superbia herds. A separate cluster analysis was conducted on the commercial sector animals only and the DeltaK illustrated that these herds had no genetic sub-structure since only one cluster could be identified. A bar plot (Fig. 3.2) shows the full assignment of individuals to the four clusters.

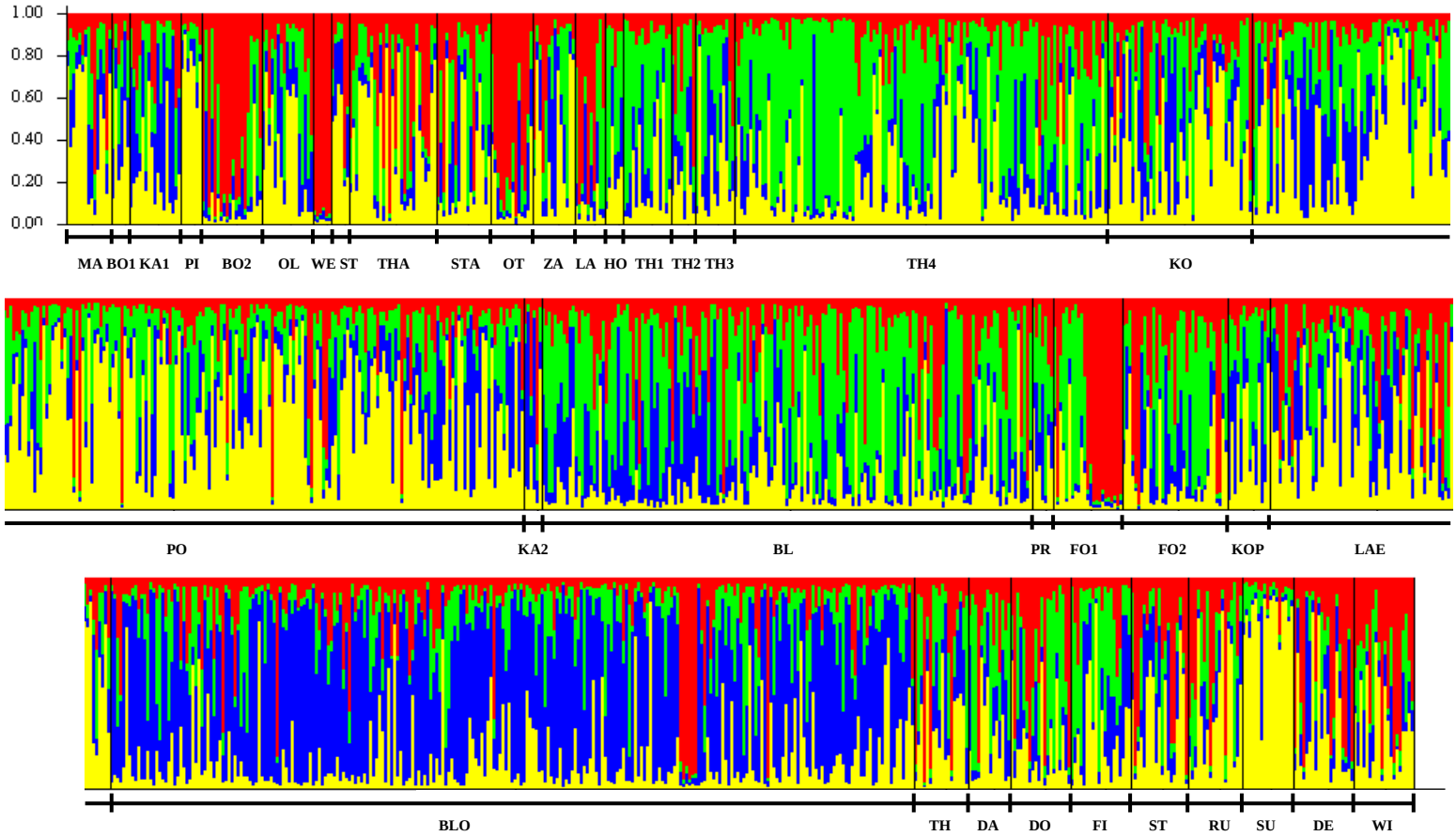

Figure 3.2 Bar plot showing proportion of membership to four genetic clusters (K = 4) identified using Structure Harvester. Cluster one (red), cluster two (green), cluster three (blue) and cluster four (yellow)

Table 3.4 Proportion of membership of each herd to each of four identified clusters (K=4)

Herd	Herd status	Inferred clusters				No. of individuals
		Cluster 1	Cluster 2	Cluster 3	Cluster 4	
MA	Stud	0.186	0.149	0.191	0.474	15
BO1	Stud	0.122	0.253	0.255	0.370	6
KA1	Stud	0.168	0.215	0.368	0.249	17
PI	Stud	0.067	0.098	0.082	0.753	7
BO2	Stud	0.610	0.206	0.103	0.081	20
OL	Stud	0.167	0.255	0.237	0.341	17
WE	Stud	0.943	0.015	0.022	0.020	6
ST	Stud	0.269	0.072	0.209	0.450	6
THA	Stud	0.332	0.195	0.083	0.390	29
STA	Stud	0.280	0.282	0.165	0.274	18
OT	Stud	0.638	0.130	0.118	0.114	14
ZA	Stud	0.191	0.217	0.232	0.359	14
LA	Stud	0.509	0.286	0.133	0.072	10
HO	Stud	0.102	0.504	0.164	0.231	6
TH1	Stud	0.084	0.522	0.180	0.213	16
TH2	Stud	0.243	0.303	0.251	0.203	8
TH3	Stud	0.119	0.386	0.250	0.263	13
TH4	Stud	0.114	0.510	0.142	0.235	124
KO	Stud	0.180	0.226	0.225	0.369	48
PO	Stud	0.126	0.215	0.198	0.461	236
KA2	Stud	0.233	0.096	0.456	0.215	6
BL	Stud	0.198	0.439	0.223	0.139	163
PR	Stud	0.177	0.380	0.229	0.215	7
FO1	Stud	0.579	0.261	0.041	0.119	23
FO2	Stud	0.287	0.372	0.172	0.169	35
KOP	Stud	0.091	0.306	0.279	0.323	14
LAE	Stud	0.225	0.173	0.217	0.384	69
BLO	Stud	0.138	0.145	0.570	0.146	267
TH	Commercial	0.295	0.251	0.192	0.262	18
DA	Commercial	0.217	0.358	0.200	0.244	14
DO	Commercial	0.351	0.323	0.130	0.197	20
FI	Commercial	0.123	0.387	0.244	0.246	20
ST	Commercial	0.357	0.138	0.209	0.295	19
RU	Commercial	0.319	0.209	0.128	0.344	18
SU	Commercial	0.059	0.074	0.066	0.801	17
DE	Commercial	0.420	0.093	0.211	0.277	20
WI	Commercial	0.433	0.171	0.116	0.281	20

Genetic differentiation between 37 selected herds in the Afrikaner cattle breed, expressed as p values from F_{ST}, is presented in Table 3.6. A total of 703 herd pair-wise combinations were performed. From these, the number of combinations with significant ($P<0.05$) differentiation after Bonferroni correction (424) outnumbered the combinations that showed no significant differences between herds (242). The Wesselsbron (WE) herd was consistently the most divergent from the remaining populations, and differed significantly from all 36 other herds. The Otjiwarongo (OT) herd also showed considerable differentiation, being significantly different from all herds except the Theunissen2 (TH2) and Fochville1 (FO1) herds.

In the AMOVA analysis (Table 3.5), virtually no variation was detected between the stud- and commercial groups, with only 0.34 % of variation attributed to differences between stud- and commercial herds. By comparison, 3.9 % of variation found was due to variation within each of these two groups. The remaining 95.8 % of variation was accounted for by differences among individuals within herds.

Table 3.5 Hierarchical distribution of overall genetic diversity in stud and commercial herds (AMOVA)

Source of variation	Sum of squares	Variance components	Percentage variation (%)
Among group	15.168	0.00912	0.33513
Among herds within groups	344.35	0.10518	3.8662
Within herds	7050.058	2.60621	95.79867

A dendrogram based on Nei's genetic distances (D_A) is presented in Fig. 3.3. Three main groups were identified, with both stud and commercial herds represented in each group. Several herds, namely Theunissen, Dannhauser, Hoopstad, Rustenburg, Fochville2, Winburg, Otjiwarongo, Fochville1 and Wesselsbron clustered separately from the three main groups. However, the majority of the branches in Fig. 3.3 had insignificant bootstrap support (< 50%).

Table 3.6 Pairwise genetic differentiation (F_{ST} p-values) between stud and commercial Afrikaner cattle herds. Significant differentiation between herds where p < 0.00007. Indication of degree of differentiation between two herds is presented with a + (significant differentiation) or – (no differentiation)

	MA	BO1	KA1	PI	BO2	OL	WE	ST	THA	STA	OT	ZA	LA	HO	TH1	TH2	TH3	TH4	KO	PO	KA2	BL	PR	FO1	FO2	KOP	LAE	BLO	TH	DA	DO	FI	ST	RU	SU	DE	WI
MA	*																																			
BO1	+	*																																		
KA1	+	+	*																																	
P1	-	+	+	*																																
BO2	+	+	+	+	*																															
OL	+	-	+	+	+	*																														
WE	+	+	+	+	+	+	*																													
ST	+	-	-	-	+	-	+	*																												
THA	+	-	+	-	+	+	+	+	*																											
STA	-	-	-	-	-	-	+	-	-	*																										
OT	+	+	+	+	+	+	+	+	+	+	*																									
ZA	-	-	-	-	+	-	+	-	+	-	+	*																								
LA	+	+	+	+	-	+	+	+	+	-	+	+	*																							
HO	-	-	+	-	+	-	+	-	-	-	+	-	+	*																						
TH1	+	+	+	+	+	-	+	+	+	-	+	-	+	+	*																					
TH2	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	*																				
TH3	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	*																			
TH4	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	*																		
KO	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	*																	
PO	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-	+	+	*																
KA2	+	-	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	*															
BL	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	-	+	+	+	-	*														
PR	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	*													
FO1	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	*												
FO2	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	-	+	+	+	-	+	-	+	*											
KOP	+	-	-	+	+	-	+	-	+	-	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	*										
LAE	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	+	+	*									
BLO	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	-	-	+	+	+	-	+	-	+	+	+	+	*								
TH	-	-	+	-	+	-	+	+	-	-	+	-	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	*							
DA	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	+	+	-	-	-	-	+	+	+	-	+	-	*						
DO	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	*					
FI	+	-	+	+	+	-	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	-	-	+	*				
ST	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	-	-	+	-	+	-	+	-	+	+	+	-	+	-	-	+	+	*			
RU	-	-	+	-	+	-	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	*		
SU	+	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*	
DE	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*	
WI	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*

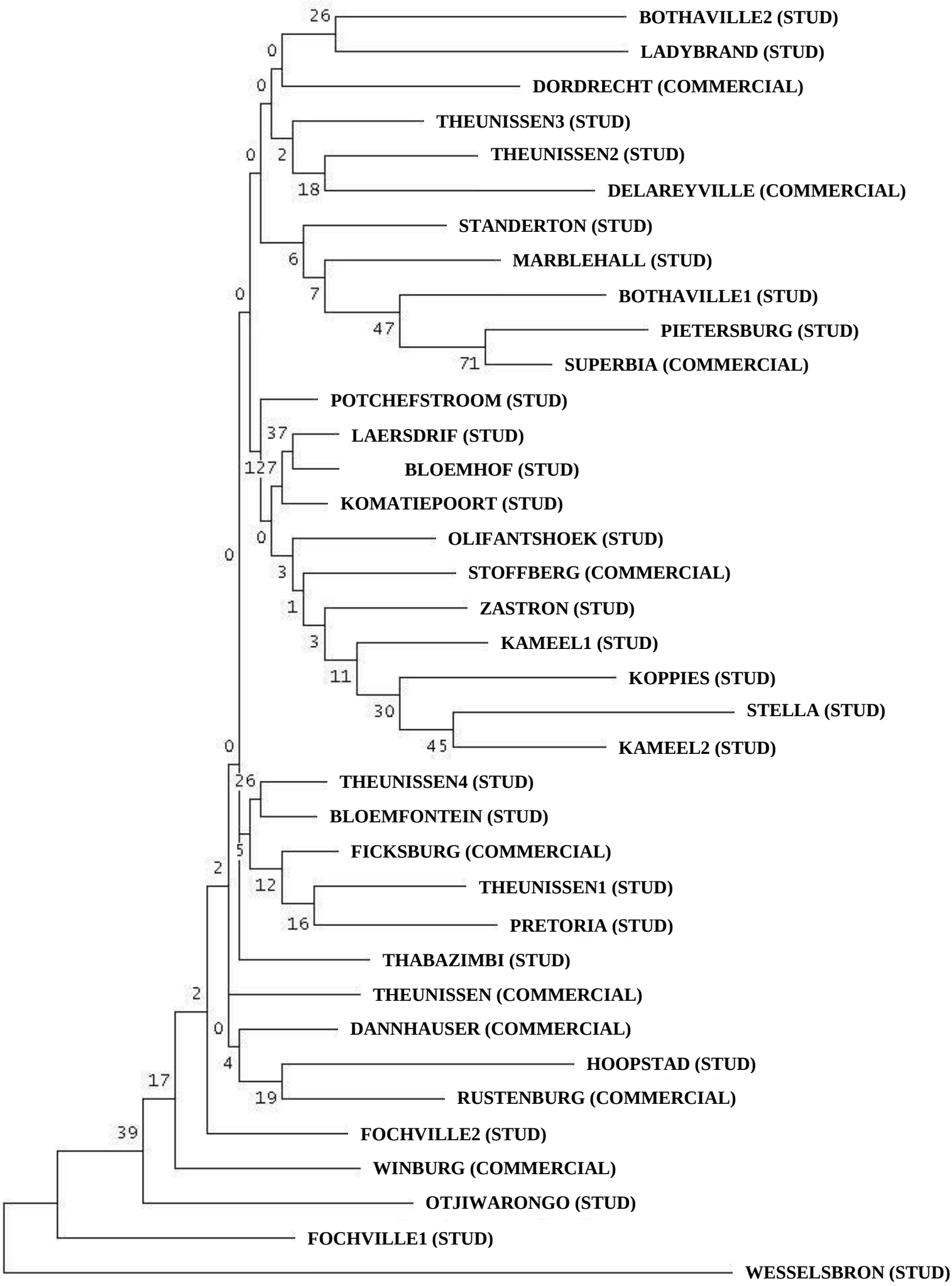

Figure 3.3 Unrooted neighbour-joining tree constructed from D_A distances showing the relationships between 37 Afrikaner cattle herds. Genetic distances are based on pooled data of 9 loci

3.5 Discussion and conclusion

This study represents the first attempt to determine levels of genetic variation in an indigenous South African cattle breed, the Afrikaner. In comparison with indigenous breeds in other countries, the Afrikaner had lower genetic diversity (as measured by Hz) compared to values reported for Indian cattle (Panday *et al*., 2006), Bhutan cattle (Dorji *et al*., 2003), European breeds (Cañón *et al*., 2001), Chinese indigenous breeds (Zhang *et al*., 2007), Ethiopian indigenous breeds (Dadi *et al*., 2008) and Mozambican cattle breeds (Bessa *et al*., 2009). However, the Afrikaner had higher Hz values than Czech Simmental, Polish Red, German Red (Čítek *et al*., 2006) and Angus (Carruthers *et al*., 2011) cattle breeds.

Table 3.7 Mean unbiazed heterozygosity estimates for selected cattle breeds found in the literature

Reference	Country	Breed	Mean unbiazed Heterozygosity
Čítek *et al*., 2006	Polish	Polish Red	0.415
Čítek *et al*., 2006	German	German Red	0.431
Čítek *et al*., 2006	Cezch	Czech Simmental	0.506
Carruthers *et al*., 2011	International	Angus	0.525
Current study	South Africa	Afrikaner (commercial)	0.557
Current study	South Africa	Afrikaner (stud)	0.567
Current study	South Africa	Afrikaner (combined)	0.568
Cañón *et al*., 2001	France	Aubrac	0.611
Carruthers *et al*., 2011	Canada	Angus	0.630
Peelman *et al*., 1998	Belgium	Belgian Blue	0.650
Bessa *et al*., 2009	Mozambique	Landim	0.661
Panday *et al*., 2006	India	Kenkatha	0.685
Bessa *et al*., 2009	Mozambique	Angone	0.688
Dadi *et al*., 2008	Ethiopian	Fogera	0.691
Peelman *et al*., 1998	Belgium	Red Pied	0.710
Panday *et al*., 2006	India	Kherigarh	0.717
Dorji *et al*., 2003	Bhutan	Siri west	0.718
Dadi *et al*., 2008	Ethiopia	Danakil	0.723
Zhang *et al*., 2007	China	Wannan	0.748
Brennenman *et al*., 2007	USA	American Brahman	0.750
Zhang *et al*., 2007	China	Zaobei	0.801
Teneva *et al*., 2005	Bulgaria	Grey	0.860

Heterozygosity values for the commercial- and stud herds were also estimated separately. The values of 0.571 ± 0.067 and 0.567 ± 0.07 respectively differ only slightly and it can be concluded that the contributions of the stud- and commercial herds to overall genetic diversity within the Afrikaner population are approximately equal.

Based on the popularity of the Afrikaner cattle breed, it was used as a dam line for crossbreeding purposes to create six other composite breeds. These breeds use the Afrikaner for its adaptive capabilities to extreme environmental conditions and disease resistance. This alternative use of Afrikaner individuals caused a major decline in the numbers of pure bred Afrikaner animals being born each year due to a large number of animals primarily being used to develop the other breeds. A decrease in the number of stud- and commercial herds followed and fewer herds meant an increase in inbreeding and a potential decreased amount of available genetic resources for adaptation purposes. However, it is important to view the results from a heterozygosity perspective, where the Afrikaner has still an abundance of variation that resides within both stud- and commercial herds.

Factors contributing to heterozygote deficiency in populations are inbreeding, null alleles, population substructure, genetic hitchhiking (Nei, 1987) and restricted gene flow (Frankham *et al*., 2010). Higher inbreeding levels were expected in the Afrikaner breed; however the F-statistics (F_{IT} and F_{IS}) calculated for the breed demonstrated low levels of inbreeding with an excess of heterozygous individuals within individual herds as well as in the whole population.

Results from STRUCTURE and genetic distances indicated a comparative lack of genetic differentiation among populations in both the commercial- and stud herds, and with clusters showing exchange between the two groups. This was anticipated, but not to this extreme. One possible explanation is the movement of stud bulls to commercial herds without taking cognisance of genetic diversity. Therefore, genetic material selected in stud herds can be found in commercial herds as well and caused the erosion of genetic distances between stud- and commercial herds. Another possible explanation is the decline in the number of commercial- and stud herds in the population.

Differentiation estimates showed that the Otjiwarongo herd is significantly different from 35 of the other herds. The most probable explanation for the uniqueness of the Otjiwarongo herd is the geographical placement of the herd, which is situated in the north of Namibia. Although this Southern African country borders on the North Western part of South Africa, it

can be assumed that contemporary gene-flow between the Namibian and South African herds may be lacking or intermittent.

The genetic distance values and associated dendrogram (Fig. 3.3) showed no clear patterns describing genetic relationships between the herds studied. All branches in the neighbour joining tree showed weak bootstrap support. Therefore, geographical distribution did not play a role in the clustering of herds. The small amount of between-herd genetic differentiation that does exist, as detected from AMOVA values, can possibly be attributed to genetic drift or local adaptation to the environment. The AMOVA values (Table 3.5) also confirm an almost complete lack of differentiation between stud and commercial herds, with less than 1 % (0.34 %) of the variation attributed to differences between these two categories of Afrikaner cattle herds. These results can possibly be the results of relatively low rates of drift between the herds, despite the assumed infrequent exchange of breeding animals between breeders of both stud- and commercial herds.

The principle findings of this study are high genetic diversity within but low genetic distances between stud- and commercial herds of the Afrikaner cattle breed. The current study showed genetic variability levels within the Afrikaner cattle are higher than expected, with comparatively high heterozygosity values in both the stud- and commercial herds, even though the magnitude of variability is slightly less than literature values reported for other breeds (Table 3.7). The study also demonstrated that there is no difference between stud- and commercial herds in the breed, which was unexpected. This can be seen as a positive result that can be used in future breeding programs.

CHAPTER 4

PEDIGREE ANALYSIS OF THE AFRIKANER CATTLE BREED

(The Afrikaner Cattle Breeders' Society of South Africa)

4.1 Abstract

Pedigree information on the Afrikaner cattle breed was analysed to determine the mean level of inbreeding (*F*), effective population size (N_e) and generation intervals. A total of 244714 pedigree records recorded from 1940 until 2011were used in the analysis. The effective number of founders (f_e), effective number of ancestors (f_a) and mean average relatedness (*AR*) were also calculated. The average inbreeding coefficient calculated was 1.83 % and the effective population size computed using the increase in the individual rate of inbreeding was estimated at 167.54. A total of 84138 animals were inbred to some degree. When the entire dataset was considered, a total of 34.4 % proportion of animals was therefore inbred to some degree. The effective number of founders and effective number of ancestors was 288 and 226 respectively. The mean average relatedness was 0.44 % and the number of complete generations was 6. In addition, the average generation interval for the whole population was calculated as 6.554 ± 3.883 years. Pedigree analysis on the Afrikaner cattle population yielded levels of inbreeding that appear to be at acceptable and at manageable levels. By implication, the result of the low inbreeding levels is the high effective population size.

4.2 Introduction

Inbreeding causes a reduction in genetic variability which in return is the basis of inbreeding depression affecting fitness-related traits such as fertility (Falconer & Mackay, 1996; Croquet *et al*., 2007; González-Recio *et al*., 2007; Panetto *et al*., 2010). The reduction of genetic variability in beef cattle has been researched extensively (Swiger *et al*., 1961; Burrow, 1993; Santana *et al*., 2010). However, inbreeding in the indigenous cattle breeds of Southern Africa have not yet received enough attention. Vahlsten (2004) emphasized the importance of monitoring the level and rate of inbreeding within a population over time. Inbreeding can be defined as the probability that two alleles positioned on the same locus on a chromosome, are identical by descent (Bourdan, 2000). Pedigree analysis is therefore, an important tool to determine the rate and level of inbreeding and genetic variability in a breed. In addition, inbreeding increases the frequency of homozygous genotypes and consequently decreases the frequency of heterozygote genotypes in a population. The size of the Afrikaner cattle breed has decreased to a much smaller number of herds and animals, compared to former numbers.

Therefore, it was imperative that a pedigree analysis be conducted on the Afrikaner to ensure the conservation of this indigenous South African breed.

One method to ensure the future existence of the Afrikaner breed is through the maintenance of genetic variability. Genetic variation is important for the adaptive potential of a population and will be utilized when faced with extreme environmental changes such as global warming. In addition, genetic variation will ensure a capacity for long-term response to selection pressures, either through artificial or natural selection (Frankham *et al*., 2003).

Quantitative genetics that is based on pedigree analysis is an alternative to the use of molecular genetic markers, such as microsatellite markers, to determine the level of genetic variability and inbreeding within a livestock breed. Genetic analysis studies based on pedigree information has been researched extensively within livestock species such as dairy cattle (Maignel *et al*., 1996; Maiwashe *et al*., 2006), beef cattle (Gutiérrez *et al*., 2003; Bouquet *et al*., 2011; Steyn *et al*., 2012), horses (Moureaux *et al*., 1996; Vicente *et al*., 2012) and sheep (Huby *et al*., 2003; Maiwashe & Blackburn, 2004). The aim of the current study was to use pedigree data from the Afrikaner cattle breed to examine the pedigree structure of the breed, to determine the levels of inbreeding, effective population size (N_e) and generation intervals. Parameters derived from the probability of gene origin i.e. effective number of founders (f_e) and effective number of ancestors (f_a) were also investigated.

4.3 Materials and methods

4.3.1 Data

Pedigree analysis of the Afrikaner cattle breed was conducted using ENDOG v 4.8 software (Gutiérrez & Goyache, 2005). A total of 244714 individuals were used in this analysis, which included records from 1940 until 2011. ENDOG can be used to conduct several different types of analysis on pedigree data. This includes demographic and genetic analysis that allows the monitoring of both changes in population structure and genetic variability. The primary functions provided by ENDOG are: firstly, computing individual inbreeding (*F*) (Wright, 1931); secondly, computing average relatedness (*AR*) coefficients (Gutiérrez *et al*., 2003; Goyache *et al*., 2003) and thirdly, information about pedigree completeness. The software is also useful to estimate parameters in population genetics (described by Boichard *et al*., 1997) indicating the number of ancestors that describe genetic variability and to

explain the genetic importance of herds (proposed by Robertson, 1953 and Vassallo *et al*., 1986).

4.3.2 Inbreeding

Rates of inbreeding (*F*) were calculated using the regression of applicable values on the year of birth (Meuwissen & Lou, 1992).

The increase in inbreeding (Δ*F*) was calculated as:

$$\triangle F = \frac{Ft-(Ft-1)}{1-(Ft-1)}$$

where F_t and F_{t-1} = the average inbreeding at the i_{th} generation

Inbreeding coefficient (F_i) can be defined as the probability that an animal is homozygous for an allele at a given locus. It was calculated for each animal within the dataset according to the methods of Meuwissen & Lou's (1992). According to Lacy *et al*. (1996) it is possible to estimate partial or incomplete inbreeding coefficients for a number of ancestors or founders.

The individual increase in inbreeding **(**ΔF_i) was estimated using the individual inbreeding coefficient (F_i) as described by Gonzalez-Recio *et al*. (2007) and modified by Gutiérrez *et al*. (2009). With the use of $\boldsymbol{\Delta F_i}$ it is possible to distinguish between two animals with the same inbreeding coefficient, but with a different number of generations in which the inbreeding took place (González-Recio *et al*., 2007; Gutiérrez *et al*., 2009).

The individual increase in inbreeding was calculated by:

$$\triangle F_i = 1 - \sqrt[t-1]{1 - F_i}$$

where F_i = individual coefficient of inbreeding

and *t* = equivalent complete generations

The software ENDOG v 4.8 was used to calculate *t* (Gutiérrez & Goyache, 2005).

4.3.3 Effective Population size (N_e)

This parameter can be defined as the number of animals used for breeding which would lead to an increase in inbreeding if the contribution of all breeding animals were equal. ENDOG software calculates N_e as:

$$N_e = \frac{1}{2\triangle F}$$ (Cervantes *et al*., 2008)

where ΔF = increase in inbreeding

4.3.4 Average relatedness (AR)

AR is the probability that an allele belongs to a specific animal when it was randomly selected from the total population within the pedigree. *AR* is useful to estimate the effective population size of the founder population.

4.3.5 Pedigree Completeness

This parameter indicates the degree of depth of the pedigree. It was measured in complete generation equivalents (CGE). CGE can be defined as the degree of pedigree information of an animal.

Three traced generations is computed by ENDOG for each animal in the pedigree:

(i) Fully traced generations – defined as the individuals separating the progeny of the furthest generation, which is where the second generation of ancestors of the specific individual are identified. Generation 0 are the founders of the population, which are the ancestors whose parents are both unknown.

(ii) Maximum amount of generations traced – defined as the amount of generations which separates the individual from its outermost ancestor.

(iii) Equivalent complete generations – estimates the pedigree of each individual as the sum over all known ancestors of the terms $(1/2)^n$. The amount of generations which separates the individual from each identified ancestor is represented by n (Maignel *et al*., 1996; Boichard *et al*., 1997).

4.3.6 Generation Intervals

The generation interval was calculated as the average age of the parents at the birth of their offspring that replaced them (Falconer & MacKay, 1996).

4.3.7 Effective number of founders (f_e)

The parameter f_e can be defined as the number of contributing founders that would be expected to produce the same level of genetic diversity as the population under investigation. For a given number of founders, the more equal the genetic contribution of each founder, the greater the number of effective founders that will be identified.

The effective number of founders was calculated by:

$$f_e = \frac{1}{\sum_{k=1}^{f} q_k^2}$$

where f_e = actual number of founders

and q_k = probability that founder *k* is the origin of the gene

4.3.8 Effective number of ancestors (f_a)

Boichard *et al*. (1997) defined f_a as the minimum number of ancestors (including both founders and non-founders) which demonstrates the complete diversity of the population being studied. The information provided by f_e is complemented by f_a given that these parameters account for the losses of the genetic variation that was produced by the uneven number of reproductive animals which produced bottlenecks (Gutiérrez & Goyache, 2005). The effective number of ancestors was calculated by:

$$f_a = \frac{1}{\sum_{j=1}^{a} q_j^2}$$

where f_a = actual number of ancestors

and q_j = genetic contribution of ancestor *j*

4.4 Results

Parameters characterizing the genetic variability of the Afrikaner breed are presented in Table 4.1. The original dataset contained 244714 animals. The reference population, where both parents are known for an animal, contained 203821 animals. The number of founder animals, where one or both parents are unknown, were 396.16 animals. The effective number of

founders (f_e) and ancestors (f_a) were 288 and 226, respectively. A total of 21263 ancestors were recorded which contributed to the reference population. A total of 201 ancestors explained 50 % of the genetic variability of the reference group. The mean inbreeding value was calculated as 1.83 % with an average relatedness of 0.44 %. The founder genome equivalents (f_g) was 396.16. Lacy (1989) defined f_g as similar to f_e, where the number of contributing founders that would be expected to produce the same level of genetic diversity as the population currently being studied, under the condition that loss of alleles did not occur and all founders were represented as equal.

Table 4.1 Parameters characterizing the probability of gene origin in the Afrikaner cattle breed

Item	Total
Original dataset	244714
Reference population (animal with both parents known)	203821
Number of founder animals (animal with one or more unknown parent)	396.16
Number of ancestors contributing to reference population	21263
Effective number of founders (f_e)	288
Effective number of ancestors (f_a)	226
Number of ancestors explaining 50 % of genetic variability	201
Number of founder herds in reference population (f_h)	528
Effective number of founder herds for the reference population	35.2
Mean average relatedness (*AR*) (%)	0.44
Maximum number of generations	4.42
Number of complete generations	1.86
Number of equivalent generations	2.81
Founder genome equivalent (f_g)	396.16
Effective population size (N_e)	167.54
Mean inbreeding (%)	1.83

The different levels of inbreeding of the animals studied are presented in Table 4.2. There are four animals which are more than 40 % inbred. A total of 84138 animals were inbred to some degree, therefore 160576 animals showed no or low levels of inbreeding. Highly related matings within the dataset of the Afrikaner are presented in Table 4.3. A total of 67 matings between full sibs (brother and sister) were recorded, which presents 0.03 % of the population under study. Furthermore, mating between half sibs (half-brother and sister or brother and half-sister) was recorded at a total of 8607 cases, which amounts to 3.52 %. Finally, mating between parent and offspring (mother and son or father and daughter) were recorded at 1.17 % of the records within the dataset, accounting for 2856 animals.

Table 4.2 Levels of inbreeding in the Afrikaner breed

Number of animals (N)	Level of inbreeding (%)
4	>40
230	30 - 39.9
3144	20 - 29.9
11666	10 - 19.9
13140	6 - 9.9
35820	1-5.9

Table 4.3 Highly related matings noted in the current study

Related matings	N and %
Matings between full sibs	67 (0.03%)
Matings between half sibs	8607 (3.52%)
Matings parent-offspring	2856 (1.17%)

Only six complete generations were documented (presented in Table 4.4). Generation 0 started with 40893 animals and at generation six only 61 animals were recorded. The mean inbreeding ranged between 1.02 % and 4.75 %. As expected, an increase in inbreeding was observed. At generation one, only 8.4 % of the 59943 animals were to some degree inbred; with large spikes in the percentages of animals which were inbred between the first and second complete generations and between the second and third complete generations; and with a gradual increase from there onwards. No specific trend described trends in the effective population size (N_e) from one generation to the next.

Table 4.4 Number of complete generations; number animals (N); Mean inbreeding per generation (Mean F); percentage inbred animals per generation; mean inbreeding coefficient of percentage inbreeding animals; Effective population size (N_e)

Complete generations	N	Mean *F* (%)	Inbred animals (%)	Avg *F* for inbred animals (%)	N_e
0	40893	0			
1	59943	1.02	8.44	12.8	48.6
2	68734	2.43	37.14	6.57	35
3	46609	2.72	61.59	4.43	168.7
4	23670	3.10	85.11	3.65	128.2
5	4808	3.71	96.38	3.85	80.4
6	61	4.75	98.36	4.86	45

The increase in inbreeding and the average relatedness (*AR*) per generation are illustrated in Fig. 4.1. A maximum of 14 generations were detected. Generation 0 to two showed no levels of inbreeding (orange line). From generation two to six there was a steady increase in inbreeding of ± 1 % per generation. Onwards until generation 12, inbreeding levels were constant with minimal to no increase of inbreeding for the six generations. From generation 12 to 14, a sharp increase in inbreeding was observed. Average relatedness per generation (green line) followed a similar trend compared to the levels of inbreeding. Generations 0 to three displayed a minimum to no increase in relatedness. From generation three to generation six, a gradual increase was observed. From there on, a constant relatedness level was observed with a slight increase from generation 13 to 14.

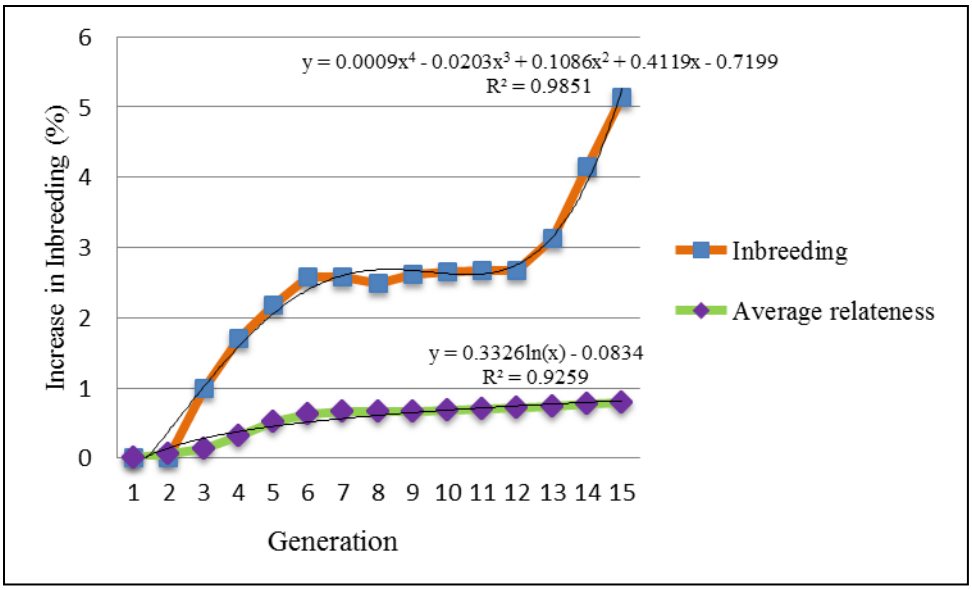

Figure 4.1 Increase in inbreeding and average relatedness per generation

Pedigree completeness for the dam (female) and sire (male) lines are presented in Fig. 4.2. The first ancestral generation, calculated from all the animals in the dataset, were 87 % complete. The second ancestral generation for dam and sire lines were on average 69.9 % and 73.9 % complete, respectively. For the third generation the pedigree completeness decreased to the lowest percentage, at 48.4 %. The average generation interval for the whole population was calculated as 6.554 ± 3.883 years. Furthermore, the generation interval estimated for the four different gametic pathways were as follows: dam to son: 6.712 ± 3.653 years; dam to daughter: 6.615 ± 3.514 years; sire to son: 6.507 ± 4.704 years and sire to daughter: 6.489 ± 4.146 years.

The mean inbreeding level per year for whole pedigree data and the mean inbreeding level per year for inbred animals are presented in Fig. 4.3. The proportion of animals that were inbred in 1970 were very low, therefore they're average inbreeding level were high. While the opposite happened in 2011 a larger proportion of animals were inbred, this therefore translated to a lower inbreeding level. From 1970 to 1979, the inbreeding level for all animals showed an increase of 0.94 %; onwards from 1980 until 2011 all inbreeding levels were above 1 %. A stable increase in inbreeding levels was visible from 1978 to 1988 and from there on constant levels followed. The year with the highest inbreeding level was 1996, with an average of 2.79 %. Along with the number of inbred animals increasing, the number of

generations of inbred animals also increased; therefore, the level of inbreeding effectively decreased over time. Consequently, the level of inbreeding for the inbred animals showed a constant decrease as the number of generations used in analysis increased. The number of animals used for determining the level of inbreeding per year is presented in Fig. 4.4. In 1981, there was a sharp increase in the number of animals used in the analysis for both inbred animals and all pedigree data used. The reason for this sharp rise in the number of animals was driven by the society to register more animals.

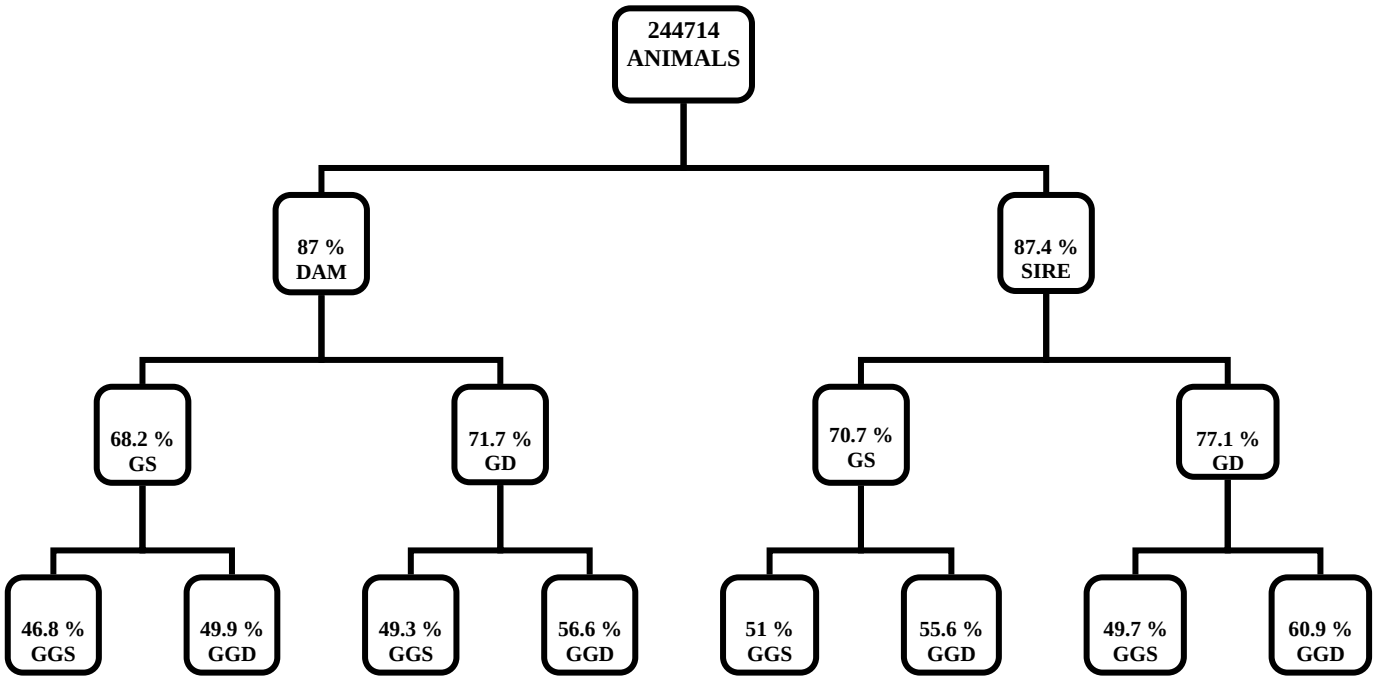

Figure 4.2 Level of pedigree completeness of three generations in the Afrikaner dataset

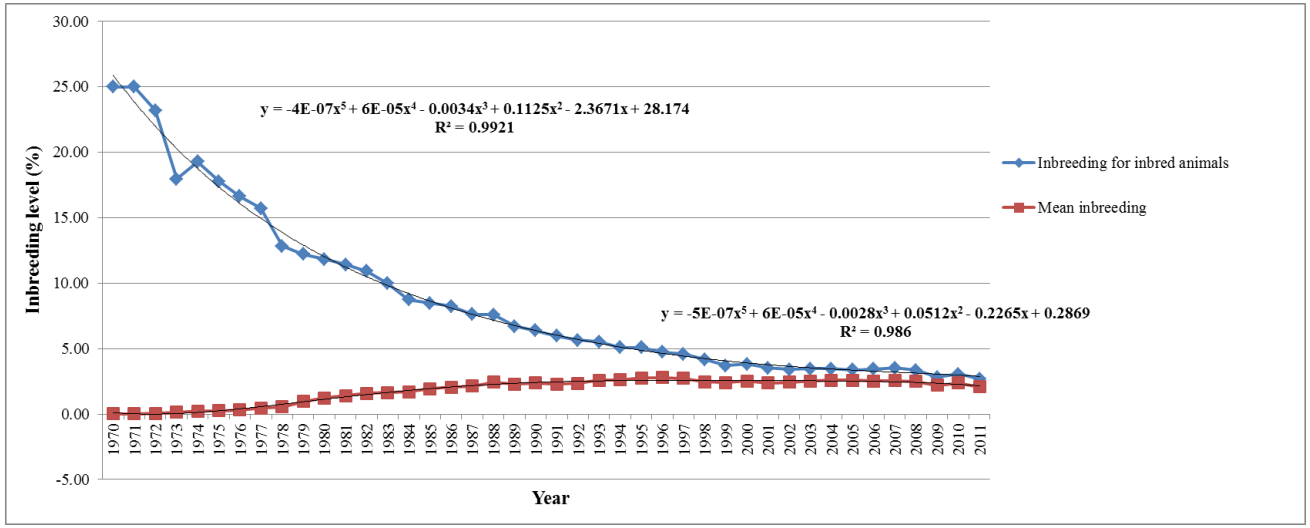

Figure 4.3 Mean inbreeding level per year for whole pedigree data and mean inbreeding level per year for inbred animals

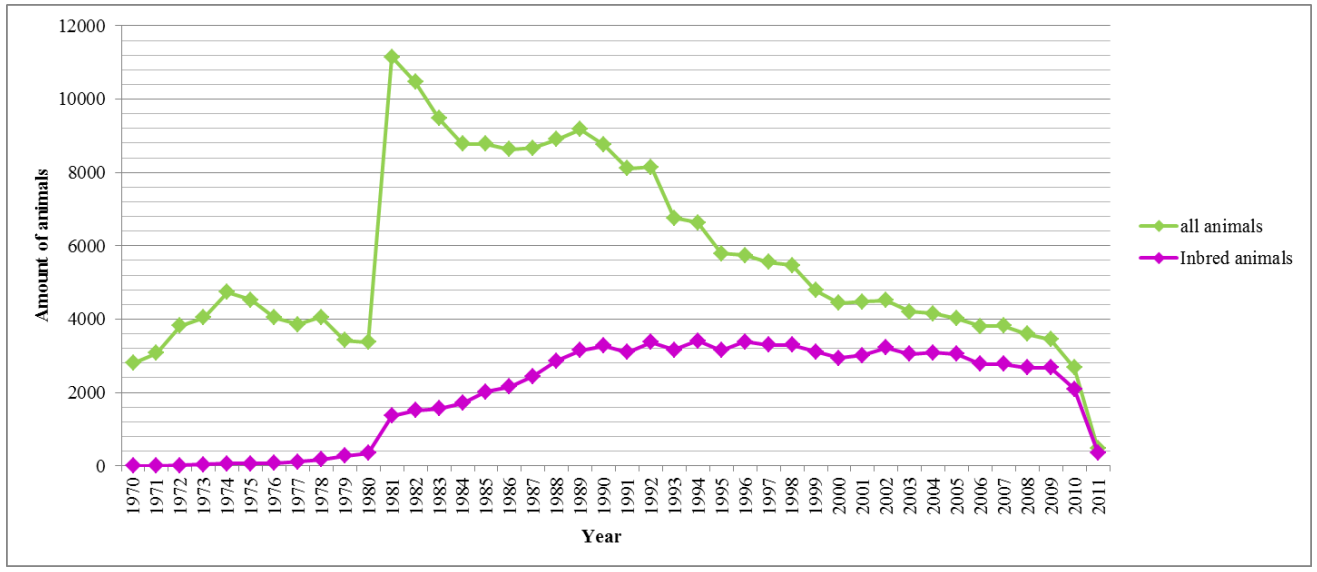

Figure 4.4 Number of animals used to calculate the level of inbreeding per year for whole pedigree data and inbred animals

4.5 Discussion and conclusion

When the entire dataset was considered, a total of 34.4 % of animals was to some degree inbred. The average inbreeding coefficient for the whole population was 1.83 %. The N_e estimate of the Afrikaner is large if it is compared to some international breeds. McParland *et al*. (2007) estimated N_e sizes of 64, 127, and 75 for Irish Herefords, Simmental, and Holstein-Friesian, respectively, whereas that of Italian beef cattle breeds were in the range from 122-138 (Bozzi *et al*., 2006). Estimates of N_e for Danish dairy cattle populations ranged from 49-157 (Sørenson *et al*., 2005). The value of N_e for the current study is 167.54, calculated as described by Cervantes *et al*. (2008). This estimate is identical to that of the Nguni that was estimated to be 168 (Matjuda, 2012). The N_e reported here is greater than the 50 to 100 recommended by the FAO (1998) and within the 25 to 255 range of effective population size that was suggested as being critical for maintaining fitness by Meuwissen and Woolliams (1994). Furthermore, ENDOG software was also used also computed the effective size via two other methods, namely complete generations and equivalent generations, with values of 61.67 and 87.48 obtained. When comparing the different estimates given for N_e, the level of pedigree completeness and the depth of the pedigrees should be taken into account. The effective population size is generally not stable and changes over time through several different events that may occur. These include: (i) change in average inbreeding in the population; (ii) change in number of known parents and number of progeny of a parent; and (iii) the change in generation interval. In addition, should the rate of inbreeding in the population increase, the N_e could possibly decrease to a value lower than the critical effective population size value (Fair *et al*., 2012). The Afrikaner cattle population under study have acceptable levels of the estimated effective population size.

The comparatively low levels of inbreeding observed in Afrikaner cattle could be due to random mating processes or crossbreeding that were induced within the herds. A project involving introgression into the Afrikaner breed was initiated by a number of breeders. Bonsmara bulls were used for mating with pure Afrikaner cows, *inter alia* to increase genetic diversity. This project may have contributed to keep inbreeding in the Afrikaner population at low levels. As an additional consequence, the relatedness between the animals decreased.

Table 4.5 Mean inbreeding coefficient (*F*) of different cattle breeds

Reference	**Country**	**Breed**	**(*F*) (%)**
Santana Jr *et al*., 2012	Brazil	Bonsmara	0.26
Steyn *et al*., 2012	South Africa	Drakensberger	0.93
Hunlun (unpublished)	South Africa	Bonsmara	0.95
Gutiérrez *et al*., 2003	Spain	Morucha	1.22
Santana Jr *et al*., 2012	Brazil	Marchigiana	1.33
Gutiérrez *et al*., 2003	Spain	Alistana	1.53
Gutiérrez *et al*., 2003	Spain	Sayaguesa	1.73
Current study	South Africa	Afrikaner	1.83
Gutiérrez *et al*., 2003	Spain	Pirenaica	2.97

It is important to also compare pedigree analysis results between different breeds of cattle (Table 4.5). The South African Bonsmara (F=0.95) and Drakenberger (F=0.93) breeds, which are also indigenous, displayed lower inbreeding levels than the Afrikaner (F=1.83). From Table 4.5 it was clear that only one breed, Pirenaica (F=2.97) displayed a higher inbreeding level than the Afrikaner.

Pedigree analysis on the Afrikaner cattle population yielded levels of inbreeding that appear to be at acceptable and manageable levels, especially when compared to international breeds such as the Holstein-Friesian. The result of the low inbreeding levels confirms high effective population size.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

(Lené Pienaar)

Although microsatellite markers have been used to determine the genetic diversity in indigenous South African sheep-, goat- and poultry breeds, this study is the first attempt to make use of microsatellite markers to determine the genetic diversity in an indigenous South African cattle breed, namely the Afrikaner. Furthermore, it was also the first study to combine genetic markers and pedigree analysis to estimate the genetic variability within an indigenous South African cattle breed. The objectives of the study were to estimate genetic diversity and inbreeding levels within the breed. All objectives of the study were completed. This data can ultimately be utilized to improve and preserve the genetic resources that the breed offers to the agricultural sector of South Africa.

The three prior hypotheses, namely: (i) low levels of genetic diversity will be observed in the Afrikaner breed; (ii) a significant component of the overall genetic diversity resides in commercial as opposed to stud herds; and (iii) the current level of inbreeding present in this cattle breed are higher than the level globally accepted for cattle; were all rejected. Starting with the first hypothesis, a moderate to high degree of unbiased heterozygosity (Hz) was observed within the breed, with an average Hz value of 0.568 ± 0.067. In comparison with indigenous breeds in other countries, the Afrikaner breed had lower genetic diversity (as measured by Hz) compared to values reported for Indian cattle (Panday *et al*., 2006), Bhutan cattle (Dorji *et al*., 2003), European breeds (Cañón *et al*., 2001), Chinese indigenous breeds (Zhang *et al*., 2007), Ethiopian indigenous breeds (Dadi *et al*., 2008) and Mozambican cattle breeds (Bessa *et al*., 2009). However, the Afrikaner had higher Hz values than Czech Simmental, Polish Red, German Red (Čítek *et al*., 2006) and Angus (Carruthers *et al*., 2011) cattle breeds. Secondly, when heterozygosity values for the commercial- and stud herds were estimated separately, the values were 0.571 ± 0.067 and 0.567 ± 0.07, respectively. Evidently, these values differ only marginally. Therefore, it can be concluded that the contributions of the stud- and commercial herds to overall genetic diversity within the Afrikaner population are equal.

The third hypothesis relating to the levels of inbreeding was also rejected due to the mean inbreeding (*F*) level of 1.83 % and the effective population size (N_e) of the Afrikaner population being 167.54. The average inbreeding coefficient was much lower and the effective population size was larger compared to globally accepted reference values. The South African Bonsmara breed and Drakensberger which is also indigenous to South Africa had lower inbreeding (*F*) levels than the Afrikaner. Other breeds such as Morucha, Alistana,

Sayaguesa from Spain (Gutiérrez *et al*., 2003) and Marchigiana from Brazil (Santana Jr *et al*., 2012) also had marginally lower *F* values than the Afrikaner.

A comparison between the inbreeding coefficients estimated from the microsatellite data as $F_{IT,}$ and pedigree data as *F,* was also conducted. The estimates of F_{IT} and *F* were on average 1.7 % and 1.83 % respectively. The difference between these two values was significantly lower than expected, notwithstanding the difference in methods applied to acquire these results. Furthermore, the study populations used within each of these two approaches also differed significantly, with only a few herds overlapping. The F_{IT} and F_{IS} values could be considered to be the more accurate estimate, since all microsatellite data used were factual values. In contrast, results from the pedigree analysis take into account all data available, even when per generation data and other pedigree data were not complete. Nevertheless, results from both approaches indicated that low levels of inbreeding are present within the Afrikaner cattle population.

Lower than expected levels of genetic differentiation and genetic distances among the 37 herds were observed. This can however be accounted for by the fact that breeding bulls from stud herds are frequently brought into commercial herds, thus compensating for a superficial lack of gene flow between the two types of herds found in the overall Afrikaner cattle population.

In the current study 11 microsatellite markers, proposed by ISAG, was tested. These markers were mostly informative and high levels of polymorphism were observed, although they were specifically developed for the use on European cattle breeds and not necessarily Southern African Sanga breeds. Only two of the markers were not useful with only a minimal number of animals showing polymorphism. Nevertheless, it is proposed that alternative or at least additional molecular markers should be developed for application in future studies, that will allow analyses more tailored to use for Sanga type cattle, when conducting parentage verification and genetic diversity testing.

The study of genetic differentiation yielded informative results on the genetic relationship between stud- and commercial herds. The study demonstrated that only 0.34 % of overall diversity could be attributed to differentiation among these two cattle populations. Furthermore, the geographical distribution of the herds played no role in any of the patterns of relatedness that were observed, thus exchanges of animals between populations appear to

have a genetic homogenising effect in Southern Africa. The implication is that there are no real genetic differences between stud- and commercial herds. Consequently, a commercial animal can be registered as a stud animal (for example in the F1 generation), provided complete pedigree data is available for that particular animal.

It is important to note that in recent years the use of single nucleotide polymorphism (SNP) markers has gained popularity as the primary method for genome-wide studies, as well as genetic diversity studies (Edea *et al*., 2013). Several recent studies have demonstrated the usefulness of SNPs (McKay *et al*., 2008; Negrini *et al*., 2008; Lin *et al*., 2010; Edea *et al*., 2013) in cattle diversity studies. SNPs have several advantages over microsatellite markers; such as low mutation rate, more reliable genotyping and data interpretation (Krawczak, 1999) and automation (Lindblad-Toh *et al*., 2000). Therefore, in future studies describing the genetic diversity in livestock breeds the use of SNP markers should be considered.

It is proposed that genetic diversity studies, such as the current study, are continued in the future to observe any genetic changes that may occur in the Afrikaner population (and other indigenous breeds). This will allow for timely implementation of management and conservations plans to ensure the future preservation of indigenous South African cattle breeds.

From the combined use of both genetic markers and pedigree analysis, it was observed that moderate to a higher than expected genetic diversity levels, low genetic differentiation and distances between herds occur within the Afrikaner population. In addition, low levels of inbreeding were also observed. The current study provided useful results than can be utilized by farmers and breeders' societies of Southern African cattle breeds. The results of this study indicate that there is sufficient variation within the Afrikaner breed of cattle that will allow it to expand into the diverse markets that exist both within and beyond the boundaries of South Africa, especially in the era of climate chance. The results also indicate that the breed is in a favourable position to continue countering the undesirable effects of inbreeding. Further investigations need to be carried out to examine the performance history of, and genetic trends for performance and reproductive traits in the breed. In conclusion, these results can be used to ensure that the Afrikaner cattle population will take its rightful place in the South African beef cattle industry, and unique indigenous genotypic and phenotypic characteristics of the breed should be reemphasized.

SUMMARY

This study was a first attempt to use microsatellite markers to determine the genetic diversity in an indigenous cattle breed, namely the Afrikaner. It was also the first study to combine genetic markers and pedigree analysis to estimate the genetic variability within an indigenous cattle breed. The objectives of the study were to estimate genetic diversity and inbreeding levels within the breed and to utilize the results to preserve and ultimately improve the genetic resources offered by the breed.

A total of 1214 stud animals (representing 28 herds) and 166 commercial animals (nine herds) from different geographical areas within and adjoining South Africa were included in this study. Animals were genotyped at the two major animal molecular laboratories in South Africa, with both using the same standardized 11 marker microsatellite set. Estimates of genetic diversity did not support the hypothesis of significant loss of genetic diversity in the Afrikaner breed. Heterozygosity estimates ranged from 0.737-0.456 within individual populations, with an overall heterozygosity estimate of 0.568 for the Afrikaner breed. Assignment methods (based on STRUCTURE software) revealed a real structure consisting of four genetic populations (K=4). No consistent pattern of significant differentiation between stud- and commercial herds could be identified.

Pedigree information, based on a total of 244714 recorded animals from 1940 to 2011, were analysed to determine the mean level of inbreeding (F), effective population size (N_e), generation interval, effective number of founders (f_e), effective number of ancestors (f_a) and average relatedness (AR). The average inbreeding coefficient calculated was 1.83% and the effective population size computed using the increase in the individual rate of inbreeding was estimated at 167.54. A total of 84138 animals (34.4%) were inbred to some degree. The effective numbers of founders and ancestors were 288 and 226 respectively, with an average relatedness of 0.44% and with results confirming a total of six complete generations. The average generation interval for the whole population was calculated as 6.554 ± 3.883 years.

It is concluded that a moderate to high degree of variation is still present within the Afrikaner cattle breed, despite the recent decline in numbers of this indigenous breed. Levels of inbreeding appear to be at acceptable and at manageable levels. The current study provided

results than can be utilized by farmers and breeders' society to conserve the Afrikaner and develop the breed to its full potential.

Key words: Average relatedness, *Bos taurus africanus*, effective number of ancestors, effective population size, genetic differentiation, heterozygosity, inbreeding, indigenous cattle breeds, microsatellite markers, pedigree analysis.

OPSOMMING

Hierdie studie verteenwoordig ‘n eerste poging om die genetiese diversiteit in ’n inheemse beesras, naamlik die Afrikaner, met mikrosatelliet merkers te bepaal. Dit is ook die eerste studie wat beide genetiese merkers en stamboomdata gekombineer het om die genetiese diversiteit van ’n inheemse beesras te bepaal. Die doelstellings vir die studie was om die genetiese diversiteit en vlakke van inteling van die ras te bepaal en sodoende die resultate te gebruik om die ras te bewaar en uiteindelik die genetiese hulpbronne wat die ras bied te benut.

In totaal is 1214 stoet diere (verteenwoordigend van 28 kuddes) en 166 kommersiële diere (nege kuddes) vanaf verskillende geografiese areas van binne en aangrensend aan Suid Afrika in die studie ingesluit. Stoetdiere is gegenotipeer by twee van die vernaamste diere molekulêre laboratoriums in Suid Afrika, wat albei dieselfde gestandardiseerde 11 mikrosatelliet merkerstel gebruik het. Beramings van die genetiese diversiteit het nie die hipotese ondersteun wat voorspel het dat daar beduidende verliese aan genetiese diversiteit in die Afrikaner ras was nie. Heterosigositeit waardes het gewissel tussen 0.737-0.456 binne individuele kuddes, met ‘n algehele heterosigositeit waarde van 0.568 vir die Afrikaner ras. Toewysings metodes (gebasseer op STRUCTURE sagteware) het ’n werklike struktuur wat bestaan uit vier genetiese bevolkings (K=4) getoon. Geen konstante patroon van beduidende differensiasie tussen stoet- en kommersiële kuddes kon geïdentifiseer word nie.

Stamboom inligting, gebaseer op ’n totaal van 244714 aangetekende diere vanaf 1940 tot 2011, is geanaliseer om die gemiddelde vlak van inteling (*F*), effektiewe bevolkingsgrootte (N_e), generasie interval, effektiewe aantal stigters (f_e), effektiewe aantal voorouers (f_a) en die gemiddelde verwantskap (*AR*) te bepaal. Die berekende gemiddelde inteelkoëffisiënt was 1.83% en die effektiewe bevolkingsgrootte bereken deur gebruik te maak van die verhoging in die individuele tempo van inteling was 167.54. ’n Totaal van 84138 diere (34.4%) was tot ’n mate ingeteel. Die effektiewe aantal stigters en voorouers was 288 en 226 onderskeidelik, met ’n gemiddelde verwantskap van 0.44%. Die resultate het ’n totaal van ses volledige generasies bevestig. Die gemiddelde generasie interval vir die hele bevolking was bepaal as 6.554 ± 3.883 jaar.

Dit is vasgestel dat ’n matigde tot hoë vlak van genetiese variasie steeds teenwoordig is in die Afrikaner beesras, ten spyte van die onlangse verlaging in die aantal diere van dié inheemse ras. Die vlakke van inteling is ook op aanvaarbare en hanteerbare vlakke. Die huidige studie het resultate gelewer wat deur telers en telersgenootskap benut kan word om die Afrikaner te bewaar en die ras te ontwikkel tot sy volle potensiaal.

Sleutelwoorde: *Bos taurus africanus*, effektiewe aantal voorouers, effektiewe populasiegrootte, genetiese differensiasie, gemiddelde verwantskap, heterosigositeit, inheemse beesrasse, inteling, mikrosatelliet merkers, stamboom analiese.

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APPENDIX A

ALLELE FREQUENCIES FOR EACH LOCUS

Allele frequencies for locus BM1824

Locus	Herds.....																																				
BM1824	**OL**	**HL**	**JC**	**FO**	**FR**	**GVZ**	**FJ**	**KC**	**TK**	**NV**	**KV**	**CV**	**AJH**	**PG**	**AA**	**CC**	**IC**	**MC**	**SA**	**PP**	**CI**	**FN**	**BK**	**PDC**	**PDCII**	**DPB**	**SJJ**	**DKJD**	**TH**	**DA**	**DO**	**FI**	**ST**	**RU**	**SU**	**DE**	**WI**
178	0.07	*	*	*	0.08	*	0.33	*	0.04	0.08	*	0.07	*	*	*	*	*	*	*	0.00	*	0.02	0.14	0.09	0.07	*	0.04	0.01	0.03	*	*	*	0.05	0.03	*	*	*
180	0.77	0.17	0.69	0.93	0.35	0.50	0.58	0.58	0.46	0.56	0.50	0.61	0.35	0.58	0.44	0.57	0.42	0.41	0.60	0.52	0.42	0.37	0.43	0.48	0.49	0.39	0.43	0.53	0.53	0.25	0.28	0.33	0.47	0.56	0.41	0.68	0.73
182	*	0.25	0.16	*	0.25	0.09	*	*	*	0.08	0.23	*	0.20	*	0.16	*	0.19	0.06	0.06	0.06	0.08	0.15	0.14	0.07	0.10	0.04	0.05	0.15	0.08	0.07	0.08	0.13	0.03	0.03	*	0.05	0.05
186	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	0.03
188	*	*	*	*	0.08	*	*	0.17	*	*	*	*	*	*	*	*	*	*	*	0.00	0.08	*	*	0.02	0.01	*	*	0.01	*	*	*	*	*	*	*	*	*
190	*	*	*	*	*	*	*	*	0.07	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	*	*	*	*	*	*	*	*	*	*
192	0.17	0.58	0.16	0.07	0.25	0.41	0.08	0.25	0.43	0.28	0.27	0.32	0.45	0.42	0.41	0.43	0.39	0.53	0.33	0.42	0.42	0.45	0.29	0.35	0.33	0.57	0.47	0.31	0.36	0.68	0.65	0.55	0.45	0.39	0.59	0.28	0.20
194	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Allele frequencies for locus BM2113

Locus	Herds.....																																				
BM2113	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCII	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
121	0.20	*	0.13	*	0.15	0.12	*	*	0.14	0.17	0.04	0.25	0.30	0.33	0.09	0.25	0.08	0.16	0.14	0.18	*	0.28	0.14	0.22	0.16	*	0.13	0.25	0.14	0.14	0.13	0.25	0.05	0.14	0.06	0.03	0.20
123	*	*	*	*	*	*	*	*	0.02	*	0.04	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
125	*	0.17	*	*	0.03	*	*	*	*	*	0.04	*	0.10	*	*	0.06	*	0.04	0.02	*	*	0.01	*	*	0.13	*	0.01	0.02	*	0.11	*	*	0.08	0.08	*	0.15	0.18
127	*	*	0.16	*	*	0.03	0.33	*	*	*	*	0.07	*	*	*	*	*	*	0.02	0.01	0.25	0.04	*	*	0.04	0.07	0.04	0.12	0.03	0.04	*	0.03	0.08	*	0.03	0.03	0.03
129	*	*	*	0.14	0.03	0.06	0.25	*	0.09	0.17	0.11	0.07	0.05	*	0.44	0.06	0.31	0.30	0.10	0.07	0.08	0.20	0.14	0.13	0.09	0.14	0.04	0.06	0.11	0.07	0.35	0.25	*	*	0.03	0.03	0.15
131	0.03	*	*	*	*	*	0.08	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	0.11	0.04	*	*	*	0.03	*	*	*	0.03	*	*	*	*
133	0.47	0.67	0.56	0.50	0.45	0.47	0.17	0.92	0.05	0.44	0.46	0.36	0.40	0.17	0.31	0.56	0.54	0.33	0.46	0.59	0.58	0.35	0.36	0.33	0.40	0.61	0.53	0.38	0.22	0.39	0.40	0.23	0.61	0.33	0.47	0.75	0.13
135	*	*	0.06	0.21	0.50	*	*	*	0.04	0.17	0.07	0.14	*	*	0.03	0.06	0.04	0.03	0.05	0.05	0.08	0.04	0.07	0.15	0.06	0.18	0.04	0.01	0.06	0.11	0.08	0.10	0.11	0.17	0.15	0.03	0.18
137	*	*	*	*	0.03	*	*	0.08	0.02	*	*	*	*	*	*	*	*	*	*	0.03	*	0.00	*	0.04	*	*	0.01	0.01	0.03	*	*	*	*	0.03	*	*	*
139	0.27	0.17	0.09	0.14	0.25	0.27	0.08	*	0.16	0.03	0.25	0.11	*	0.08	0.09	*	0.04	0.06	0.16	0.03	*	0.04	0.07	*	*	*	0.10	0.07	0.19	0.11	*	0.10	0.05	0.17	0.27	*	0.15
141	0.03	*	*	*	0.25	0.06	0.08	*	0.04	0.03	*	*	*	0.42	0.03	*	*	0.08	0.05	0.04	*	0.04	0.21	0.02	0.07	*	0.09	0.08	0.19	0.04	0.05	0.05	*	0.08	*	*	*
143	*	*	*	*	*	*	*	*	*	*	*	*	0.15	*	*	*	*	*	*	*	*	*	*	*	0.01	*	*	*	*	*	*	*	*	*	*	*	*

Allele frequencies for locus ETH10

Locus	Herds.....																																				
ETH10	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCI	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
209	*	*	0.09	0.07	0.10	*	*	*	0.10	*	0.04	*	0.20	0.25	0.19	*	0.08	0.17	0.08	0.21	*	0.02	0.07	0.17	0.14	*	0.13	0.01	0.06	0.07	*	0.03	0.03	0.14	0.35	0.03	0.13
211	*	0.08	*	0.29	0.05	0.18	0.08	0.30	0.10	*	0.07	*	*	*	0.03	*	*	0.05	0.08	0.06	*	0.10	0.21	0.02	0.03	*	0.08	0.03	0.19	0.04	0.03	0.08	0.03	*	0.03	*	0.13
213	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.02	*	*	0.01	*	0.03	*	*	*	0.03	*	*	0.03	*
215	*	*	*	*	*	*	*	*	*	0.03	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.03	*	*	*	*
217	*	*	0.12	*	0.03	0.06	0.58	0.10	0.05	*	0.04	0.18	0.05	0.08	*	*	0.04	0.05	0.02	0.04	0.10	0.02	*	0.24	0.10	0.04	0.07	0.05	0.03	0.04	0.10	*	0.03	0.06	*	0.03	0.20
219	0.47	0.33	0.09	0.29	0.45	0.18	0.08	0.20	0.31	0.36	0.43	0.18	0.30	0.33	0.16	0.43	0.29	0.21	0.29	0.19	*	0.33	0.07	0.20	0.24	0.07	0.16	0.12	0.22	0.39	0.55	0.23	0.34	0.17	0.15	0.45	0.25
221	0.53	0.58	0.71	0.36	0.38	0.59	0.25	0.40	0.43	0.61	0.43	0.64	0.40	0.33	0.63	0.57	0.58	0.52	0.51	0.50	0.90	0.53	0.64	0.33	0.49	0.89	0.54	0.72	0.47	0.46	0.33	0.68	0.53	0.64	0.47	0.45	0.30
223	*	*	*	*	*	*	*	*	*	*	*	*	0.05	*	*	*	*	*	0.01	*	*	*	*	0.02	*	*	0.01	0.07	*	*	*	*	*	*	*	0.03	*
233	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Allele frequencies for locus INRA23

Locus	Herds.....																																				
INRA23	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCII	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
192	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.03	*	*	*	*	*	*	*	*
194	*	*	*	*	*	*	*	*	0.07	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
196	0.40	0.67	0.47	0.79	0.15	0.62	0.33	0.67	0.52	0.50	0.54	0.57	0.40	0.75	0.50	0.38	0.40	0.53	0.53	0.65	0.50	0.47	0.86	0.41	0.59	0.29	0.59	0.59	0.58	0.64	0.68	0.60	0.63	0.53	0.53	0.45	0.43
198	0.13	0.08	0.35	*	0.23	0.24	*	0.17	0.16	*	0.04	0.25	0.05	*	0.06	0.38	0.30	0.17	0.18	0.14	0.33	0.19	*	0.22	0.17	0.46	0.16	0.19	0.17	0.07	0.05	0.13	0.18	0.14	*	0.50	0.10
200	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*
202	*	*	*	*	0.10	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*
206	*	*	*	*	*	*	0.33	*	0.02	*	*	0.04	*	0.08	*	0.06	*	0.01	0.01	0.00	*	0.03	*	0.02	0.03	*	*	0.00	0.03	0.04	*	*	*	0.08	*	*	0.05
208	0.30	0.25	0.15	0.14	0.05	*	0.33	0.17	0.19	0.33	0.17	0.11	*	0.17	*	0.13	0.15	0.09	0.21	0.12	0.08	0.11	*	0.07	0.07	*	0.20	0.06	0.08	0.11	0.13	0.13	0.16	0.11	0.29	0.05	0.38
210	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.07	*	*	*	*	*	*	*	*	*	*	*	*	*
214	0.17	*	0.03	*	0.48	0.15	*	*	0.05	0.17	0.25	0.04	0.55	*	0.44	0.06	0.15	0.19	0.07	0.09	0.08	0.21	0.14	0.22	0.14	0.21	0.05	0.16	0.11	0.14	0.15	0.15	0.03	0.14	*	*	0.05
220	*	*	*	0.07	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	0.04	*	*	*	*	*	*	*	*	0.18	*	*

Allele frequencies for locus SPS115

Locus	Herds.....																																		
SPS115	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCII	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
242	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
246	*	*	0.03	*	*	*	*	0.17	*	*	*	0.04	*	*	*	*	*	*	*	0.01	0.17	0.01	*	0.04	0.06	*	0.02	0.02	0.03	*	*	*	0.03	*	*	*	*
248	1.00	1.00	0.97	1.00	0.76	1.00	1.00	0.83	1.00	0.97	0.96	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	0.83	0.98	1.00	0.74	0.79	1.00	0.94	0.98	0.86	0.93	0.88	1.00	0.97	0.97	1.00	0.90	0.93
249	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
250	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	*	0.00	*	*	0.01	*	0.04	0.00	*	0.04	*	*	*	0.03	*	*	*
252	*	*	*	*	*	*	*	*	*	0.03	*	*	*	*	*	*	*	*	*	*	*	*	*	0.02	0.01	*	*	*	*	*	*	*	*	*	*	*	*
254	*	*	*	*	0.13	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	0.01	*	0.02	0.01	*	*	*	*	*	0.03	*	*	*	*	0.05	0.03
256	*	*	*	*	*	*	*	*	*	*	0.04	*	*	*	*	*	*	*	*	0.00	*	*	*	0.04	0.03	*	*	0.00	0.03	0.04	0.03	*	*	*	*	*	0.05
258	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.08	*	*	*	*	*	*
260	*	*	*	*	0.11	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	0.13	0.09	*	*	*	0.08	*	*	*	*	*	*	0.05	*

Allele frequencies for locus TGLA53

Locus	Herds.....																																				
TGLA53	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCI	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
154	*	*	*	*	*	*	*	*	*	*	*	*	0.05	*	*	*	*	0.02	*	0.00	*	0.06	*	0.02	0.01	*	*	0.00	*	*	*	*	*	*	*	0.03	*
158	*	*	*	*	0.17	*	*	*	*	*	0.04	*	*	*	*	*	*	*	*	*	*	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
160	0.37	0.58	0.82	0.21	0.47	0.62	0.25	0.75	0.36	0.56	0.61	0.57	0.70	0.50	0.34	0.40	0.61	0.36	0.54	0.57	0.83	0.44	0.64	0.20	0.29	0.54	0.48	0.52	0.53	0.61	0.63	0.45	0.50	0.44	0.32	0.40	0.04
162	*	*	*	*	*	*	*	*	*	*	0.07	*	*	*	*	*	*	*	0.02	0.00	*	*	*	0.02	*	*	0.01	0.04	*	0.04	0.08	*	*	*	*	0.05	0.13
164	*	*	*	*	*	0.03	0.08	*	*	*	*	*	*	*	*	*	*	*	0.01	0.00	*	*	*	*	*	*	*	*	*	*	*	*	0.05	*	*	*	*
166	*	*	*	*	0.03	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	0.02	0.01	*	*	*	0.03	*	*	*	*	*	*	*	*
168	*	*	*	*	*	*	0.42	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	0.15	0.01	*	*	0.00	*	*	*	*	*	*	*	*	*
170	*	*	*	*	*	*	*	*	*	*	*	*	0.05	*	*	*	*	*	*	*	*	*	*	0.07	*	*	0.01	*	*	*	*	*	*	*	*	*	*
172	*	*	*	*	*	*	*	*	*	*	*	*	0.10	*	*	*	*	0.02	*	0.00	*	0.00	*	0.04	*	*	*	*	*	*	*	*	*	*	*	0.03	*
174	*	*	*	*	0.03	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.03
176	0.43	0.25	0.15	0.64	0.22	0.18	0.08	0.08	0.52	0.36	0.14	0.29	0.05	0.17	0.47	0.40	0.33	0.39	0.26	0.23	0.17	0.24	0.36	0.24	0.34	0.14	0.34	0.24	0.33	0.25	0.28	0.33	0.21	0.31	0.59	0.40	0.38
178	0.07	*	0.03	0.07	0.03	*	*	*	0.03	*	*	0.04	0.05	0.08	*	0.10	0.06	0.02	0.02	0.03	*	0.02	*	*	0.01	*	0.04	0.01	*	*	0.03	0.05	*	0.03	*	0.05	*
180	*	*	*	*	0.03	*	*	*	0.05	*	0.07	*	*	*	0.06	*	*	0.09	0.05	0.00	*	0.10	*	0.11	0.07	0.14	*	0.05	*	*	*	0.08	*	0.06	*	0.03	0.03
182	0.13	0.17	*	0.07	*	0.18	*	0.17	0.03	0.08	*	0.11	*	0.08	0.13	0.10	*	0.08	0.04	0.15	*	0.07	*	0.11	0.13	0.18	0.09	0.11	0.11	0.07	*	0.10	0.18	0.14	0.09	*	0.10
184	*	*	*	*	*	*	0.17	*	*	*	0.07	*	*	*	*	*	*	0.00	0.05	*	*	*	*	*	0.01	*	0.03	0.02	*	*	*	*	0.05	0.03	*	*	*
186	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.03	*
188	*	*	*	*	*	*	*	*	*	*	*	*	*	0.17	*	*	*	0.01	*	*	*	0.05	*	*	0.07	*	*	0.00	*	0.04	*	*	*	*	*	*	*
192	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	*	0.02	0.03	*	*	*	*	*	*	*	*	*	*	*	*
198	*	*	*	*	0.03	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Allele frequencies for locus TGLA122

Locus	Herds.....																																				
TGLA122	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCII	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
137	0.37	0.25	0.21	0.21	0.28	0.06	*	*	0.11	0.28	0.04	*	0.05	0.25	0.03	0.25	0.15	0.29	0.24	0.19	0.08	0.14	0.21	0.09	0.13	0.04	0.22	0.28	0.31	0.21	0.08	0.18	0.16	0.11	0.21	0.23	0.05
141	*	*	*	*	*	*	*	*	*	*	0.04	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.03	*
143	0.50	0.75	0.53	0.79	0.25	0.53	0.67	0.58	0.46	0.56	0.21	0.71	0.50	0.58	0.75	0.50	0.65	0.38	0.53	0.58	0.50	0.46	0.64	0.48	0.50	0.68	0.52	0.54	0.42	0.64	0.48	0.50	0.55	0.42	0.68	0.53	0.68
145	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	*	*	*	*	*	*	*	*	*
147	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.02	*	*	*	0.00	*	*	*	*	*	*	*	*	*
149	0.03	*	0.12	*	0.15	0.29	0.17	0.42	0.20	0.11	0.07	0.21	0.30	0.08	0.06	0.19	0.15	0.27	0.13	0.18	0.33	0.18	0.07	0.17	0.21	0.29	0.16	0.07	0.11	0.04	*	0.15	0.11	*	0.03	0.15	0.15
151	0.10	*	0.12	*	0.28	0.12	0.08	*	0.23	0.06	0.50	0.07	0.10	0.08	0.16	0.06	*	0.06	0.10	0.04	0.08	0.21	0.07	0.17	0.14	*	0.09	0.05	0.14	0.11	0.35	0.18	0.18	0.47	0.09	0.05	0.13
153	*	*	*	*	*	*	0.08	*	*	*	*	*	*	*	*	*	0.04	*	*	0.00	*	*	*	0.07	0.01	*	*	*	*	*	*	*	*	*	*	*	*	*
155	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
157	*	*	0.03	*	*	*	*	*	*	*	0.04	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
161	*	*	*	*	*	*	*	*	*	*	0.07	*	0.05	*	*	*	*	*	*	*	*	0.00	*	*	*	*	0.01	0.02	0.03	*	0.08	*	*	*	*	0.03	*
163	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	*	*	*	*	*	*	*	*	*
167	*	*	*	*	0.05	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.03	*	*	*	*	*	*
169	*	*	*	*	*	*	*	*	*	*	0.04	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
171	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	*	*	*	*	*	*	*	*	*	0.01	*	*	*	*	*	*	*	*	*
183	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*

Allele frequencies for locus TGLA126

Locus	Herds.....																																				
TGLA126	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCII	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
111	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*
112	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
113	*	*	0.09	*	*	*	*	*	0.04	0.08	0.14	0.07	0.10	*	*	*	*	0.00	*	0.00	0.08	0.03	0.14	0.11	0.06	*	*	*	0.06	0.04	*	0.05	0.13	0.03	*	*	*
115	0.23	0.17	0.06	0.14	0.38	0.29	1.00	0.25	0.45	0.22	0.50	0.18	0.50	0.08	0.41	0.25	0.23	0.19	0.32	0.27	0.25	0.21	0.29	0.37	0.24	0.11	0.28	0.35	0.31	0.14	0.35	0.33	0.24	0.53	0.21	0.90	0.53
117	0.03	0.25	0.15	0.14	0.08	0.21	*	*	0.11	0.11	0.04	*	0.05	*	0.03	*	0.04	0.11	0.02	0.07	*	0.09	*	0.15	0.04	0.36	0.03	0.04	*	0.04	0.08	0.03	0.03	*	0.12	0.03	*
119	*	*	*	*	*	*	*	0.08	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	0.01	*	*	*	0.03	0.03	*	*	*	*	*
121	*	*	*	*	*	0.06	*	*	0.02	0.03	0.18	0.11	*	0.17	0.09	0.25	0.08	0.06	0.04	0.04	*	0.10	0.07	0.11	0.03	*	0.07	0.10	0.03	0.11	*	0.10	0.11	*	*	*	*
123	0.03	*	*	0.07	*	0.03	*	*	0.05	0.06	*	0.11	*	*	*	0.08	*	0.02	0.13	0.01	*	0.07	*	0.04	0.04	*	0.09	0.04	0.08	*	0.10	*	0.13	0.03	0.06	*	*
125	0.70	0.58	0.71	0.64	0.55	0.41	*	0.67	0.34	0.50	0.14	0.54	0.35	0.75	0.47	0.42	0.65	0.61	0.49	0.59	0.67	0.49	0.50	0.20	0.59	0.54	0.52	0.48	0.53	0.68	0.45	0.48	0.32	0.42	0.62	0.08	0.48
133	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	*	*	*	*	*	*	*	*	*
215	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.05	*	*	*	*

Allele frequencies for locus TGLA227

Locus	Herds.....																																				
TGLA227	OL	HL	JC	FO	FR	GVZ	FJ	KC	TK	NV	KV	CV	AJH	PG	AA	CC	IC	MC	SA	PP	CI	FN	BK	PDC	PDCII	DPB	SJJ	DKJD	TH	DA	DO	FI	ST	RU	SU	DE	WI
77	0.23	0.42	0.44	0.33	0.37	0.24	0.25	0.42	0.47	0.33	0.25	0.39	0.40	0.50	0.41	0.44	0.50	0.38	0.33	0.33	0.42	0.43	0.64	0.15	0.29	0.29	0.32	0.24	0.39	0.54	0.60	0.53	0.21	0.25	0.41	0.30	0.45
79	0.57	0.50	0.47	0.58	0.42	0.53	0.08	0.50	0.21	0.33	0.04	0.46	0.25	0.33	0.59	0.13	0.38	0.28	0.50	0.31	0.42	0.33	0.14	0.13	0.16	0.39	0.35	0.40	0.33	0.18	0.23	0.28	0.45	0.50	0.47	0.30	0.23
81	-	-	-	0.08	*	0.06	0.58	0.08	0.07	*	0.21	*	0.10	*	*	*	*	0.06	0.03	0.10	0.08	0.02	*	0.33	0.04	0.11	0.08	0.03	*	0.04	0.03	0.05	0.11	*	0.12	0.05	0.03
83	*	*	*	*	*	*	*	*	*	*	*	*	0.10	*	*	*	*	*	*	*	*	*	*	*	*	*	0.01	0.01	*	*	*	*	*	*	*	0.05	*
85	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
87	*	*	*	*	*	0.06	*	*	0.02	*	0.11	*	*	0.08	*	*	*	0.02	0.02	0.02	*	0.01	0.07	0.11	0.07	0.07	0.10	0.07	0.03	0.04	0.05	*	0.11	0.08	*	0.05	0.10
89	0.13	*	0.03	*	0.03	0.06	*	*	0.03	0.06	0.11	0.07	0.05	*	*	0.25	0.13	0.13	0.05	0.17	0.08	0.10	*	0.13	0.30	0.11	0.10	0.11	0.08	0.07	0.03	0.10	0.08	*	*	0.15	0.03
91	*	*	*	*	*	*	0.08	*	0.09	*	0.07	*	*	*	*	0.06	*	0.02	0.02	0.00	*	0.04	*	*	0.03	*	0.01	0.12	0.03	0.04	0.05	0.03	0.03	*	*	*	*
93	*	*	*	*	0.16	0.03	*	*	*	0.08	0.04	*	0.05	*	*	*	*	*	*	0.00	*	0.02	*	*	*	*	*	*	*	*	*	0.03	*	*	*	*	*	*
95	*	*	*	*	0.03	*	*	*	0.05	0.06	*	*	*	*	*	0.06	*	0.00	0.01	0.00	*	0.02	*	0.04	*	*	0.01	0.00	*	*	*	*	*	*	*	0.03	*
97	*	*	0.03	*	*	0.03	*	*	0.05	0.14	0.14	0.07	0.05	0.08	*	0.06	*	0.10	0.01	0.05	*	0.04	0.14	0.11	0.11	0.04	0.01	0.02	0.08	0.04	*	0.03	0.03	0.17	*	0.05	0.18
101	0.07	*	*	*	*	*	*	*	0.02	*	0.04	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.06	0.07	*	*	*	*	*	0.03	*