



**Phenotypic and genetic characterisation of indigenous Tswana goat in
Botswana**

By

Phetogo I. Monau

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Supervisory Committee:

Prof. E. Van Marle-Köster

Department of Animal and Wildlife Sciences
University of Pretoria
Private Bag X20
Hatfield
0028
South Africa

Prof. S. J. Nsoso

Botswana University of Agriculture and Natural Sciences
Private Bag 0027
Gaborone
Botswana

Dr C. Visser

Department of Animal and Wildlife Sciences
University of Pretoria
Private Bag X20
Hatfield
0028
South Africa

Declaration

I declare that the work documented in this thesis is my own composition, and has not been previously submitted by anyone to any other institution. Specific contributions by others are acknowledged.

Phetogo I. Monau

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Preface

This thesis has been prepared under the supervision of Prof E. van Marle-Köster, Prof S.J. Nsoso and Dr C. Visser in the Department of Animal and Wildlife Sciences. It has been structured into six chapters consisting of introduction, literature review and manuscripts published or to be submitted in peer reviewed journals. Chapter one is the general introduction where the purpose of the study was justified. In Chapter two, a literature review has been presented on several aspects of goat domestication and genetic diversity of indigenous goats. Statistical measures for diversity have also been reviewed. Chapter three and four has been prepared in manuscript format according to the guidelines of the specific journals. In chapter three, the results of the first phase of the study consisting of a survey analysis of indigenous goat production in communal farming systems of Botswana were published in Tropical Animal Health Production Journal. Phenotypic and genetic characterisation of Indigenous Tswana goats was prepared as chapter four and published in South African Journal of Animal Science. In chapter five, a scientific paper titled “Genetic diversity and population structure of indigenous and commercial goat breeds based on Illumina Goat50K SNP chip” was prepared to be submitted to the journal. The last part of the thesis contains critical review, conclusions and recommendations of the research findings and has been structured in chapter six.

Peer reviewed journals:

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Dedication

To my two lovely children; Resego and Matshego Monau

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Abbreviations

AFLP	Amplified fragment length polymorphisms
AMOVA	Analysis of molecular variance
AnGR	Animal genetic resource
BL	Body Length
BP	Before Present
BW	Body weight
CHIP	Chromatin Immuno-precipitation
CV	Cross-validation
DAD-IS	Domestic Animal Diversity Information System
DNA	Deoxyribo Nucleic Acid
FAO	Food and Agriculture Organization of United Nation
F_{IS}	Inbreeding coefficients of an individual
F_{ST}	Inbreeding coefficient of sub-population
GDP	Gross Domestic Product
GLM	Generalized Linear Model
H_E	Expected heterozygosity
HG	Heart Girth
H_o	Observed heterozygosity
HVI	HyperVariable region I
HW	Height at withers
HWE	Hardy Weinberg Equilibrium
KB	Kilobytes
LD	Linkage disequilibrium
MAF	Minor Allele Frequency
mtDNA	Mitochondrial Deoxyribucleic Acid
N_e	Effective population size
PCA	Principal Component Analysis
QC	Quality control
SADC	South African Development Countries
SAS	Statistical System Analysis
SE	Standard Error
SNP	Single Nucleotide Polymorphism
STRs	Short tandem repeats

TL Tail length
WAD West African Dwarf

ABSTRACT

The aim of this study was to characterize indigenous Tswana goats in four agro-ecological regions of Botswana i.e. Southern, Central, Northwest and Ghanzi. The following specific objectives were set; description of existing goat production systems in Botswana, phenotypic and genetic characterisation of Tswana goats and investigation of population structure of indigenous and commercial goats using the Goat50K SNP panel. A survey was conducted in four agro-ecological regions to collect data on Tswana goats in smallholder farming systems and phenotypic measurements were recorded for 123 goats that included body weight (BW), body length (BL), heart girth (HG), height at withers (HW) and tail length (TL). Qualitative traits such as coat colour, horns and beard were also recorded. About 80% of the farmers kept goats for financial purposes. Goats in the Northwest region had significantly ($P<0.05$) higher HG values in all age groups compared to other regions. Goats in the Central (71.83 ± 1.18) and Northwest (69.17 ± 2.04) regions had significantly longer BL compared to the ones in the Southern (64.25 ± 2.50) region at >48 months. For genetic characterisation, hair samples from 48 phenotyped animals of Central region were collected and genotyped with Illumina Goat50K SNP chip. Genomic diversity was high (0.423 ± 0.03) with low inbreeding (F_{IS}) (0.009 ± 0.05). Additional genotypes which included Boer ($n=24$), Kalahari Red ($n=24$) and Swazi ($n=48$) were included in the analysis to get a broader regional perspective. Genetic diversity, measured as expected heterozygosity was 0.390 ± 0.01 , 0.398 ± 0.01 and 0.387 ± 0.02 for Boer, Kalahari Red and Swazi goats, respectively. Inbreeding coefficient ranged from 0.014 ± 0.06 in Boer, 0.012 ± 0.07 in Kalahari Red to 0.011 ± 0.06 in Swazi goats. The populations clustered according to geographical origin. Linkage disequilibrium (LD) for shorter intervals (0-10 kb) ranged from 0.44 to 0.56. Effective population size at 13th generation was approximately 87 for Boer, 93 for Kalahari Red, 180 for Swazi and 266 for Tswana goats. The results indicate potential improvement of Tswana goat through within breed selection and structured crossbreeding that will assist in food security and sustainable utilization.

Key words: Botswana, Communal, Genetic diversity, Indigenous goat, Production system, Smallholder, SNP Chip, Tswana

CHAPTER 1

GENERAL INTRODUCTION

Botswana is a landlocked, arid to semi-arid and sparsely populated country in Southern Africa where agriculture is the mainstay of the rural livelihoods (Wingqvist & Dahlberg, 2008). Although agriculture contributes only approximately 2.4% to the Gross Domestic Product (GDP), it is vital to the livelihood of many citizens of Botswana who operate farms for subsistence (Statistics Botswana, 2017). About 42% of Botswana's population lives in rural areas and roughly 70% of rural households rely on subsistence farming for their upkeep (MFD, 2009). Livestock production is by far Botswana's primary agricultural product and contributes more than 80%, while crop production contributes less than 20% to the agricultural GDP (Moreki *et al.*, 2010). The cattle industry is the primary sector contributing significantly to beef export to European market (Seleka & Kebakile, 2017). Small ruminants, particularly goats contribute enormously to the poor resource farmers and rural economy (Mrema & Rannobe, 1996). Goats constitute about 80% of the small ruminants in the country (Statistics Botswana, 2013) and are used as source of milk, meat, horns, skin, hair and play various functions in cultural and religious activities (Mrema & Rannobe, 1996). Their short generation interval compared to cattle, high reproductive rates, easy marketing and non-competiveness for food make them very useful to smallholder farmers (Aziz, 2010). Goats small size pose a smaller financial burden than cattle, as they are easier to acquire and less expensive to maintain, and require less capital for housing facilities (NaeemipourYounesi *et al.*, 2008). They have also shown resilience to a wide range of agro-ecological conditions with a range of climates, disease challenges and feed sources (Dzama, 2016).

There are approximately 1.6 million goats in Botswana, which represent approximately 35% of livestock kept (Statistics Botswana, 2016). The indigenous Tswana goats generally dominate the goat flocks with about 71% (Statistics Botswana, 2013). These goats are characterised as multi-coloured, medium sized with long lopping ears, short coarse hair, and are predominantly bearded and horned (Katongole, *et al.*, 1996; Nsoso *et al.*, 2004). Resource poor farmers keep these indigenous goats in all agro-ecological zones of the country under the traditional production system (Statistics Botswana, 2013). In this system, goats are not bred for any particular function but are rather kept for multiple purposes such as food, income, manure and skin, with less usage of advanced technology and purchased inputs (Gwaze *et al.*, 2009). Tswana goats are commonly known for utilizing sparse diets and their tolerance against heat, drought and disease/parasites. This enables them to cope with the adverse climatic conditions of the country (Nsoso *et al.*, 2004).

Despite the importance of goat production and indigenous Tswana goats in Botswana, the sector is neglected in terms of agricultural research and development programs. Goats are usually considered as subsistent animals of less profitable value. The government resources are biased against goat production in favour of cattle, e.g. there is annual cattle vaccination by government but not for goats (Mrema & Rannobe, 1996). Furthermore, small stock improvement programmes are disorganised, largely fragmented and do not produce a significant effect on goat productivity and farmers' livelihood (Nsoso & Madimabe, 2003). For instance, Tswana goats were previously crossbred with exotic breeds in order to increase the output of marketable products such as meat and milk (APRU, 1988). The crossbred goats were often superior with regard to growth, milk production and other production traits at the government research stations while at the smallholder levels were inferior (APRU, 1988). Lately, as initiatives of poverty alleviation and food security, the government of Botswana donates exotic and crossbred goats to poor farmers, but they still do not perform well under communal management systems (Moreki *et al.*, 2010). Such a strategy is unsustainable due to the mismatch of the breeding and management practices existing in the production system of the communal smallholder farmers (Kosgey *et al.*, 2006). There is also a general concern that farmers practise unplanned crossbreeding and breed replacement, which could threaten the existence of the adapted indigenous Tswana goat (Nsoso *et al.*, 2004). The genetic erosion of this breed will negatively affect the scope to respond to changing climates, diseases and other environmental challenges, which will subsequently threaten the possibility of improving the present and future livelihoods (Hoffmann, 2010).

Characterisation of Tswana goats is thus an important aspect for long-term intervention, as it will enhance general productivity and development of breeding strategies towards sustainable utilization, ultimately improving livelihoods (FAO, 2011). Previous phenotypic characterisation studies of Tswana goat were performed countrywide more than a decade ago (Nsoso *et al.*, 2004), these should be updated due to changes in production systems (Solkner *et al.*, 1998) and also be substantiated with genetic characterisation (FAO, 2011). A preliminary study was conducted to genetically characterise indigenous Tswana goats kept at a research station using low-density markers (microsatellite) (Maletsanake *et al.*, 2013). However, the studied population did not represent the wide variation that occurs in the country. Hence, there is a need to genetically characterise the indigenous Tswana goat population using dense markers in a representative population including the different geographical areas of Botswana.

Utilization of genomic tools for genetic characterisation can aid in the design of coherent programs for breed development and sustainable utilization. Microsatellites or short tandem repeats (STRs) were the preferred DNA markers for more than two decades due to their high levels of polymorphism and informativeness as well as relative abundance (Tian *et al.*, 2008; Fernández *et al.*, 2013; Marikar

& Musthafa, 2014). However, several limitations such as high technical demands and difficulty in merging data generated in different laboratories limited their use (Fernández *et al.*, 2013). Recently, single nucleotide polymorphism (SNPs) have replaced STRs due to their high abundance (Heaton *et al.*, 2005) in coding and non-coding areas, low mutation rate, stable inheritance patterns (Thomson *et al.*, 2000), high reproducibility (Lin *et al.*, 2013) and high throughput rate (Koopae & Koshkoiyeh, 2014). The development of the genome-wide high-density goat SNP array (Goat50K SNP chip) has provided an opportunity for investigating genetic diversity and inferring population history at a high resolution (Tosser-Klopp *et al.*, 2014). This offers an opportunity to characterise the indigenous Tswana goat population at a genetic level, which will ultimately assist in guiding policy and breeding programs to improve food security and the welfare of the farmers keeping this breed.

Aim of study

The overall objective of this thesis was to perform phenotypic and genetic characterisation of Tswana goats.

Specific Objectives

- To describe goat production systems in four agro-ecological regions of Botswana, using survey data.
- To perform phenotypic and genetic characterisation of indigenous Tswana goat in Botswana.
- To investigate genetic relatedness and admixture of the indigenous Tswana, Swazi, Boer and Kalahari Red goats using the Goat50K SNP panel.

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CHAPTER 2

LITERATURE REVIEW

Introduction

There are approximately one billion goats worldwide with the majority of goats kept in Asia (58.2%) and Africa (32.7%) and a few in Europe (1.7%), America (3.5%) and Oceania (0.4%) (FAOSTAT, 2016). Over 1000 goat breeds have been recognised all over the world and in Africa most breeds are kept in marginalised areas with low inputs, rudimentary management and poor nutrition (Rege, 1994). These breeds have acquired unique adaptive traits to survive and reproduce in these areas, and play a significant role in the sustenance of needy families, especially in rural areas (Abegaz, 2014; Mwai, 2015; Sarangi, 2018). There are several factors limiting the utilisation and conservation of these genetic goat resources that include lack of performance data, lack of breeding and selection goals, unintentional inbreeding and crossbreeding practises and socioeconomic pressures (Rege, 1994; Alemu, 2004; Kosgey *et al.*, 2006; Abegaz, 2014). In order to ensure that these breeds are not lost as a genetic resource, it is important to characterise them and develop proper breeding schemes that will facilitate sustainable utilisation. The aim of this review is to provide a general perspective on domestication and origin of goats, adaptability of indigenous goats and the importance of phenotypic and genetic characterisation of indigenous genetic resources.

Origin and domestication of goats

The genus *Capra* originated in Asia during the Pliocene period and dispersed in various locations of Eurasia and North Africa (Ropiquet & Hassanin, 2006). The progenitor for domestic goats has long been suggested to be the bezoars (*C. aegagrus*) (Zeuner, 1963). However, subsequent studies revealed contrasting hypotheses, suggesting three strains of the wild *Capra* as ancestors of domestic goats; bezoars (*Capra aegagrus*), markhors (*Capra falconeri*) and Sindh ibex (*C. aegagrus blythi*) (Meadow, 1996; Porter, 1996). It was suggested by Luikart *et al.* (2001) that there might be numerous maternal origins, however, Naderi *et al.* (2008) refuted the idea based on monophyletic and paraphyletic trees. The bezoars (*Capra aegagrus*) show the closest relationship with the modern domestic goat (*C. hircus*), which has been confirmed by phylogenetic analyses indicating the shortest branch length between the *Cytochrome b* and mtDNA control region (Takada *et al.*, 1997; Mannen *et al.*, 2001; Luikart *et al.*, 2006; Naderi *et al.*, 2007, 2008; Colli *et al.*, 2015).

According to archaeological remains at Ganj Dareh (Zagros Mountains, Iran), goats were domesticated approximately 11 000 years ago. Some other probable regions of domestication include the Indus Basin in Pakistan 9000 years ago (Sultana *et al.*, 2003), central Anatolia and the southern Levant 10 000 years ago (Horwitz *et al.*, 1999; Zeder, 2008). Studies based on mitochondrial

deoxyribucleic acid (mtDNA) have noted six divergent and highly diverse haplogroups; A (inherited by 90% of goats worldwide), B, C, D, F, and G (Naderi *et al.*, 2008). The authors stated that haplogroups A and C were domesticated in the Eastern Anatolia area and not at the Zagros and Iranian plateau areas (Naderi *et al.*, 2008). The early existence of both haplotype A and C in South Western Europe also infers that both lineages possibly ascended within similar temporal and geographic parameters (Fernandez *et al.*, 2006). Other haplotypes show regional distribution, such as haplotype B, which is dominant in Southeast Asia and some scholars hypothesised a second domestication in Asia where Haplogroup B arose (Sultana *et al.*, 2003; Joshi *et al.*, 2004; Chen *et al.*, 2005; Han *et al.*, 2010). Some authors disagreed and suggested that the haplotype has been imported from a nearby western area and transported to southeastern Asia where goat groups developed extreme bottlenecks, resulting in less disparity (Naderi *et al.*, 2008). The other haplotypes D, F, and G found at the domestication area either could have entered the domestic gene pool in the initial distribution of domestics in the Northern and Central Zagros, or were domesticated on a small-scale in this area (Naderi *et al.*, 2008). Despite the confusion and uncertainties of domestication site of goats, the analysis on mtDNA hypervariable (HVI) region of primitive DNA illustrated that the central Zagros played a significant role for domestication of goats (Mazdarani *et al.*, 2014)

After domestication, goats dispersed throughout the world via different routes. In Africa, the archaeological evidence and radiocarbon dates of bones indicate that domesticated goats were first introduced from Southwest Asia around 7000 before present (BP) (Pereira & Amorim, 2010). Three possible routes have been suggested; through the present-day Sahara Desert by overland diffusion, crossing the Peninsula or navigating along the Mediterranean coast (Mason, 1984; Smith, 1992; Hassan, 2000; Naderi *et al.*, 2008). Goats and sheep diffused quickly from the Near East into the central Sahara and the Ethiopian highlands within 6500 BP and 5000 BP (Newman, 1995; Clutton-Brock, 1999).

Climatic and environmental challenges (like high prevalence of Tsetse fly) stirred the movement of livestock keepers into the southern part of the continent (Smith, 1992). By 5500 BC, goats had moved into sub-Saharan Africa and a dwarf type was observed from that time close to Khartoum in Sudan (Mason, 1984). In Southern Africa, goats were presented shortly before and after the appearance of European settlers around the 4th to 7th centuries AD (Smith, 2000). The southern African goat population is therefore a mixture of various breeds that migrated from the northern tribes.

Adaptability of Indigenous goats

Adaptability of livestock can be described as the aptitude to survive and reproduce under distinct condition/location (Prayaga & Henshall, 2005; Baker, 2009). Smallholders farmers in the Tropics often stay in marginalised areas characterised by limited feed and water supplies, high disease

prevalence, hot, dry or humid or high altitude and cold (Mirkena *et al.*, 2010). Tropical areas are gifted with a diversity of indigenous goat breeds that have naturally grown to adapt to these harsh environmental conditions (Rege, 1994; Hoffman, 2010; Visser, 2017). These goats are tolerant to diseases, retain superior thermoregulation capacity, have the ability to exploit low quality forage and are adapted to water shortages with capacity to emit less methane (Darcen & Silanikove, 2017; Visser, 2017). To cope with drought for instance, these goats have the ability to desiccate their faeces, to concentrate their urine, to reduce evaporative water loss and to utilize their rumen as a water reservoir (Silanikove & Koluman, 2015). They also exhibit improved digestion efficiency, which is attributed to the maintenance of a spacious rumen that allows longer retention time of feed particles, ultimately leading to reduced feed intake and low body weight (Silanikove, 2000). Goats adapted to temperate and desert regions fed high quality forage reduced their feed intake by 20-30% and 50-55%, respectively, without any weight loss (Silanikove, 2000).

The adaptation to harsh environments may also be linked to morphological appearance and/or physical attributes of indigenous goats. Goat breeds associated with dense vegetation of western Africa possess short legs and small body sizes that increase body surface area, which dissipates heat efficiently in warm climates (Adeloye, 1998). The southern African indigenous goats possess large lop-ears or floppy ears, loose skin and long limbs that assist in heat resistance e.g. Nguni (Pieters *et al.*, 2009) and Tswana (Katangole *et al.*, 1996) breeds. Tswana goat also have mixed coat colour, which seems to be an adaptive characteristic to endure prominent seasonal fluctuations of light, heat and cold intensities (Katangole *et al.*, 1996).

Indigenous goats have shown to be well adapted to most local diseases in different regions. The west African Dwarf (WAD) goats have a high reproductive rate and are tolerant to trypanosomiasis (Daramola & Adeloye, 2009). The South African Boer breed has the ability to thrive on poor forage and tolerate diseases such as blue tongue, Prussic acid and to a lesser extent enterotoxaemia (Erasmus, 2000; Malan, 2000). The unselected indigenous veld goats of South Africa can also withstand diseases such as enterotoxaemia, anaplasmosis and endoparasites (Webb & Mamabolo, 2004). The information documented on adaptive traits of indigenous goats in Africa is however, inadequate. In Southern Africa, a few breeds such as Mashona, Tswana and Landim have been described in the Domestic Animal Diversity Information System (DAD-IS) (Table 2.1).

Table 2.1 Adaptive characteristics of the main indigenous goats in Southern African region (adapted from DAD-IS, 2017)

Breed Name	Geographical distribution	Description	Adaptive information
Mashona	Eastern, central and Northern part of Zimbabwe (Heavy rainfall areas)	Small East African (SEA) type. Varied colour pattern, white, mixed to black.	Tolerant to local diseases, hardiness
Matabele	Southern parts of Zimbabwe	Large framed, colour ranging from white through brown, mixed to black with long loped ears.	Well adapted to local conditions
Nguni	South Africa	Intermediate between SEA and lop-eared.	Resistant to tick borne diseases such as heartwater
Landim	Mozambique	SEA type, short-eared, small horned.	Adapted to harsh conditions of the country
Tswana	Eastern, Central and Southern parts of Botswana	Lop ears, large sized, long legs. White, black and mixed coloured	Withstand tick-borne disease (heartwater) and internal parasites, well adapted to local conditions

The adaptive traits are important assets of indigenous goats to exist in the current climate and in the face of a changing climate (Rischkowsky *et al.*, 2007; Rust & Rust, 2013; Dzama, 2016). It is therefore important to protect them and exploit their climatic resilience for the improvement of livelihoods and food security of rural goat keepers.

Conservation of indigenous goats

The underlying justification for conserving indigenous livestock is for food security in case of population growth, urbanization and economic development (Steinfeld *et al.*, 2006). It is well known that the world population will increase by more than two billion people reaching 9.15 billion by 2050 (FAO, 2012). Even though indigenous goat breeds are unlikely to be able to fulfil the rise in demand for livestock food products, they have several advantages as discussed in the previous section with potential of higher survival in extreme climates compared to European breeds (Rege *et al.*, 2011). At present many poor people are suffering from malnutrition and hunger due to deficient production and

distribution, and sufficient income to acquire food of adequate quantity and quality to fulfil their needs (FAO, 2012). The need to conserve indigenous goats has been recognised since the 1990s (FAO, 1999) and these goats have an important part to play in providing high quality protein and an income with relatively low inputs.

Indigenous goats ought to be conserved for their valuable adaptive traits that can be used through proper breeding strategies and application of molecular technologies like gene editing and gene introgression (Simm, 1998). This will provide options for genotype development relevant to climate change and cater for demands of livestock products (Kritensen *et al.*, 2015) The adaptive traits of indigenous goats can further be exploited through biological research in disease resistance and/or susceptibility (Simm, 1998) which could assist in the development of drugs and the control of diseases (Baker, 2001).

Indigenous goats are part of cultural heritage and as such deserve to be protected (Ansell, 2001). Many goat populations or ecotypes have played a vital role in certain national or regional history and/or cultural development (Zander, 2013). Historically and in particular before colonisation, different types of goats have been used for various socio-cultural purposes (Bettencourt *et al.*, 2013). As a fulfilment of socio-cultural obligations in Botswana, goats are used for paying bride wealth (*bogadi*) and to resolve conflicts (as *fine*) in the villages. Goats are given as a token of appreciation to friends, guests and family members (Rischkowsky *et al.*, 2007), and used for special ceremonies such as weddings, funerals, circumcision and ancestral appeasement (Katangole *et al.*, 1996). Goats play an important role in spiritual cleansing such as exorcism of evil spirits and are given as a sacrifice to calm down avenging spirits (Magangana *et al.*, 2015). Hence, conserving them will lay the groundwork for utilisation of the genetic resource for this purpose.

Methods of conservation

There are two approaches recommended by FAO to conserve animal genetic resources; *in situ* and *ex situ* practices (FAO, 1999). *In situ* conservation is the preferred method for long-term strategies and involves conservation of live farm animals in their usual habitat (Bayer, 2006; Gibson *et al.*, 2006). This method provides opportunities for breed development and evolution, utilisation and performance recording (Gibson *et al.*, 2006). The disadvantage of this method is that animals are vulnerable to natural disasters such as floods, drought, diseases and even wars. The animals are also at risk of selection and genetic drift that may result in unfavourable genetic changes on small populations, leading to genetic erosion over time (Pattison *et al.*, 2007). The *ex situ* method can be achieved in two ways; via cryopreservation of genetic material like semen, ova, embryo and DNA segments or via live population in designated places such as government farms, zoos and national parks (Engelsma, 2012). The cryopreserved genetic material can be used to create new lines or breeds and support populations

to overcome challenges such as genetic drift, inbreeding and genetic defects (Hiemstra *et al.*, 2006). However, breeds preserved in this manner are not always capable to adjust to new disease challenges or changes in production systems (Holt & Pikard, 1999). It is also quite expensive to maintain both live populations and bio-banks for cryopreservation.

The most practical method for conserving indigenous goats in southern Africa is through sustainable utilization on-farm or through community based conservation (Nyamushamba *et al.*, 2017). Community-based conservation combines the sustainable use of a breed with the empowerment of rural people who keep it (Mueller *et al.*, 2015). To encourage on-farm conservation, governments could offer subsidies or monetary incentives to indigenous goat breeders. However, prior to exploring conservation strategies, knowledge on effective conservation management requires breed identification, and quantification of genetic variability within and between breeds (FAO, 2000). This can be achieved through breed characterisation (Shabtay, 2015) that includes the different production systems where they are kept or farmed and phenotypic and genetic characterisation.

Characterisation of goats

Production systems

Knowledge on the production system is vital for understanding adaptive traits of livestock species (Otte & Chilonda, 2003). In Southern Africa, small ruminant production systems are categorised as commercial and traditional production systems. Most indigenous goats are kept in small-scale traditional production systems in communal areas (Gwaze *et al.*, 2009). The traditional production system is characterised by informal labour derived from family members, limited number of livestock kept per unit area and inadequate use of acquired inputs (Nsoso *et al.*, 2004a; Abegaz, 2007; Tavirimirwa *et al.*, 2013). This system is often hindered by land and water scarcity, diseases and predators (Gwaze *et al.*, 2009). The farmers do not have the means or inputs available for keeping records. There is a lack of breeding objectives and inbreeding may occur.

There are two main segments of traditional production systems; mixed crop-livestock and pastoral production systems (Abegaz, 2014). Mixed crop-livestock farming system involves cultivation of crops and rearing of livestock, including goats to sustain the economies of the livelihoods (Lungu, 2002). Livestock and crops are maintained as complementary ventures; animals till the land and provide manure for crop production while livestock feed on crop residues during dry season (Lungu, 2002). This system is commonly practised in most Southern African development countries (SADC), including Botswana (Lungu, 2002; Otte & Chilonda, 2003; Gwaze *et al.*, 2009). In Botswana, the system is predominant in the Central region, where the environmental conditions are suitable for both crop and livestock production.

Pastoral production system is based on keeping livestock only in large number of flock structures, primarily using natural veldt (Gizaw, 2011). Majority of food and income in this system are derived from livestock (Wakhungu *et al.*, 2014). This system is commonly practised in the western part of Botswana where the bulk supply of beef exported to European countries comes from. Crop production in this area is limited due to climate, soil conditions and wildlife (Mogotsi *et al.*, 2013). Goats are mostly kept by all pastoralists usually in combination with sheep and cattle, and seasonal migration with animals in search of water and pasture is common (Wakhungu *et al.*, 2014).

Phenotypic Characterisation

Phenotypic characterisation comprises of morphological description of animals and their production environment, considering the social and economic factors that affect them (FAO, 2011). The conventional description of breeds based on phenotype includes external appearance (e.g. coat colour, horns, beards, ear type and shape), linear body measurements (e.g. body length, heart girth, ear length, tail length), production traits (e.g. carcass weight, milk yield) and reproductive traits (e.g. age at first service, number of litters) (FAO, 2012). This approach can be used to identify breeds and determine the relationship between breeds or populations within the region or country (Mwacharo *et al.*, 2006).

Most of the southern African goats have been characterised as predominantly horned, with medium to broad lopped ears and males seems to be heavier than females (Table 2.2). However, phenotypic characterisation of indigenous goats has been inadequately conducted in the region. There are still many discrepancies with regard to identification of breeds and ecotypes and most populations are still identified by the ethnic group that keep them (Kanyile *et al.*, 2015). In Botswana, Tswana goats were phenotypically characterised more than a decade ago and in that study, geographical variation was observed (Nsoso *et al.*, 2004b). These results need to be updated and validated at genetic level (FAO, 2011).

Table 2.2 Phenotypic description of indigenous goats in Southern Africa

Breed	Distribution Area	Phenotypic description	Adult body weight (kg)		Reference
			Male	Female	
Pare	Tanzania	White, curved backward horns, medium size ears.	26	23.9	Nguluma <i>et al.</i> (2016)
Dodoma	Tanzania	Mostly white, straight horns pointed backwards	34	29	Madubi <i>et al.</i> (2000)
Landim	Mozambique	Backwards horns in both sexes, medium long ears, variable coat colour	50	35-40	DAGRIS,(2007)
Malawi goats	Malawi	Mixed coat colour, short hair, backward horns, sharp and pointed ears, medium size	45	32	Banda <i>et al.</i> (1993)
Mashona	Zimbabwe	Both sexes horned, short ears, mixed coat colour, short and fine hair	30	25	DAGRIS, (2007)
Matebele	Zimbabwe	Bearded, rarely horned, broad lopped ears, white and cream coat colour	50-55	30-40	DAGRIS, (2007)
Gwembe	Zambia	Multi-coloured, curved backwards horns	33	27	DAD-IS, (2017)
Tswana	Botswana	Multi-coloured, bearded, broad loped ears, medium size	35-50	42	Nsoso <i>et al.</i> (2004b)
Nguni	Swaziland, Lesotho,	Mixed coat colour, medium-sized ears, small frame, horned	40	26-34	Pieters <i>et al.</i> (2009)
Boer	South Africa	Long lop ears, white body and red-brownish head, curved backward horns	110-135	90-100	Pieters <i>et al.</i> (2009)
Kalahari Red	South Africa	Red coated with horns and beards	75	115	Pieters <i>et al.</i> (2009)
Savanna	South Africa	Predominantly white with horns and beards	-	60	Pieters <i>et al.</i> (2009)

Phenotypic characterisation is an easy and cost-effective tool for breed characterisation, but is influenced by the environment and the underlying genetic complexity (Gizaw *et al.*, 2011). Therefore, it needs to be supported with genetic characterisation to guide the final decisions on breed improvement programmes.

Genetic Characterisation

Genetic characterisation entails the description of breeds based on allelic frequencies and degree of polymorphism using a set of neutral reference markers (Toro *et al.*, 2009; Gizaw *et al.*, 2011). These markers include biochemical (protein) polymorphisms markers and molecular DNA markers. Protein polymorphic loci based on blood group type were the first markers used for genetic characterisation in animals e.g. cattle (Ibeagha-Awemu *et al.*, 2004), horses (Lippi & Mortari, 2003), sheep (Mwacharo *et al.*, 2002), goat (Pepin and Nguyen, 1994) and pigs (Adeola & Omitogun, 2012). The level of polymorphisms observed in proteins was often low, which profoundly limited their usage in genetic characterisation analysis (Hanotte & Jianlin, 2006). With new developments, other tools have been established such as polymorphic fragment size DNA markers (Vignal *et al.*, 2002). These markers have been applied in livestock genetic characterisation research, for instance restriction fragment length polymorphisms and randomly amplified polymorphic DNA in sheep (Kunene *et al.*, 2009; Hlophe, 2011) and goats (El-Gaali & Satti, 2009), as well as amplified fragment length polymorphisms (AFLP) in goats (Liu *et al.*, 2014) and sheep (Anila *et al.*, 2010).

Since the discovery of highly variable regions in the genomes, microsatellite markers have become the most frequently used fragment size markers for genetic diversity studies. Microsatellites are simple tandem nucleotide repeats, dispersed all over the genome, often in non-coding areas of the genome (FAO, 2011). The repeated unit can be a mono-, di-, tri- or tetranucleotide with di- repeats being most common in livestock. Microsatellites have a number of advantages compared to the above-mentioned genetic markers. They are highly polymorphic, easy to analyse and have co-dominant inheritance (Baumung *et al.*, 2004). They can also be easily genotyped even if there is a poor quality DNA sample (Selkoe & Toonen, 2006). Several microsatellite markers have been identified across different species of livestock, and for genetic characterisation, certain species-specific microsatellites have been recommended by FAO (FAO, 2011). In Africa, genetic characterisation of indigenous goats has been conducted based on microsatellites markers in a number of studies (Table 2.3). From the table it is clear that genetic diversity measured as heterozygosity varies between regions from relatively low (16%) to as high as 70%.

Table 2.3 Genetic diversity of African goats based on microsatellites markers.

Breeds	Geographic distribution	No of markers	MNA	H _o	H _e	F _{st}	Reference
Cabo Delgado, Maputo, Pafuri,	Mozambique	17	6.162	0.553	0.620	0.06	Garrine, (2007)

Tete.							
Abergelle, Agew, Begia-Medir, Bati, Gumuz.	Ethiopia	15	6.73	0.62	0.64	0.05	Hassen <i>et al.</i> (2012)
Djallonke, Mossi, Sahelian.	Burkina Faso	27	-	0.613	0.571	0.035	Traore <i>et al.</i> (2009)
Gubu/Bissau. Boran, Galla, Small East African.	Guinea Bissau Kenya	11 11	3.82 4.18	0.465 0.481	0.450 0.498	 0.05	Muema <i>et al.</i> (2009) Muema <i>et al.</i> (2009)
Born White, Red Sokoto, WAD.	Nigeria	11	4.85	0.518	0.528	0.05	Muema <i>et al.</i> (2009)
Gogo, Pare, Sonjo, Sukuma.	Tanzania	8	5.815	0.620	0.714	0.085	Nguluma <i>et al.</i> (2018)
Karamoja, Kigezi, Sebei, Teso.	Uganda	11	4.615	0.506	0.505	0.05	Muema <i>et al.</i> (2009)
Caprivi, Kavango, Kunene, Ovambo.	Namibia	18	-	-	0.623	0.11	Els <i>et al.</i> (2004)
Tswana. Arsi-Bale, Djallonke, Kigezi, Maasai, Maure, Mubende, Ndebele, NWH, Pafuri, WAD.	Botswana Africa	12 19	1.28 6.063	0.121 0.396	0.162 0.614	- 0.15	Maletsanake <i>et al.</i> (2013) Chenyambuga <i>et al.</i> (2004)

Baladi, Demusces, Farafra, Zaraibi.	Egypt	6	3.33	0.172	0.585	0.21	El-Sayed <i>et al.</i> (2017)
Angora, Boer, Kalahari Red, Savanna.	South Africa	17	-	0.618	0.678	-	Pieters <i>et al.</i> (2007)

MNA= mean number of alleles, Ho= observed heterozygosity, He= expected heterozygosity, WAD=West African Dwarf, NWH= North West High and F_{ST} = genetic variation of subpopulation.

Despite their popularity in genetic diversity studies, microsatellites have several disadvantages. Some of the disadvantages include their location in non-coding regions of the genome and difficulty in merging data generated in different laboratories (Beaumont & Bruford, 1999). Their genotyping and scoring are exhaustive and allele calling, null alleles and size of homoplasmy are difficult to interpret (Pariset *et al.*, 2009). This genetic tool is gradually being replaced by single nucleotide polymorphism (SNP) markers.

A SNP marker is a change on a single base in a DNA sequence, with two probable nucleotides at an assumed location (Vignal *et al.*, 2002). SNPs happens approximately once every 1 kb, within the coding and non-coding regions. For a variation to be regarded a SNP, it must happen in at least 1% of the population (Vignal *et al.*, 2002; Mburu & Hanotte, 2005). Advantages of SNP markers include minimal mutation, stable inheritance, present in the coding regions and could affect protein functions directly (Ellegren, 2004). Microsatellites can go through homoplasmy, whereas homoplasmy is almost absent in SNPs (Selkoe & Toonen, 2006). Compared to microsatellites, SNPs are more appropriate for high-throughput genetic analysis and automatic allele calling (Gama & Bressan, 2011). SNP data can also be comparable and shared among laboratories, which improves the technicality of handling huge datasets from fragment sized DNA markers across laboratories (Ellegren, 2004). Different genome-wide SNP arrays have been established for different farm animals, and their usefulness in investigation of breed diversity and structure has been illustrated in many studies (Lin *et al.*, 2010; Kanyile *et al.*, 2015; Makina *et al.*, 2014; Mdladla *et al.*, 2016).

A Goat50K SNP panel contains 53,347SNPs that were found by sequencing whole genomes and reduced representation libraries of six different goat breeds; Alpine, Boer, Creole, Katjang, Saanen and Savanna (Tosser-Klopp *et al.*, 2014). This panel has been used to study population structure and genetic diversity in South African commercial dairy and fibre goats (Lashmar *et al.*, 2016), South African indigenous ecotypes (Mdladla *et al.*, 2016), Ethiopian indigenous goats (Mekuriaw, 2016) and Zimbabwean indigenous goats (Zvinorova, 2017). Genetic diversity based on this chip ranged from 30% to 60% on different African indigenous goats (Table 2.4).

Besides genetic characterisation, geographic grouping among Angora goats from Argentina, South Africa and France has been achieved using this chip (Visser *et al.*, 2016). Mdladla *et al.* (2016) differentiated South African goat populations according to breed type and confirmed the genetic uniqueness of the feral Tankwa breed from the domesticated breeds. The introduction of the Goat50K SNP Bead Chip further permitted the investigation of several traits, such as polledness in Boer, Cashmere and Rangeland goats (Kijas *et al.*, 2013), wattles in different Swiss goat breeds (Reber *et al.*, 2015), coat colour in Saanen (Martin *et al.*, 2016), heat adaptation in Egyptian Barki goats (Kim *et al.*, 2016) and milk fat in French Alpine and Saanen goats (Martin *et al.* 2017).

Table 2.4 Genetic diversity of indigenous African goats based on Goat50K SNP panel

Population	N	Country	H _O	H _E	F _{IS}	Reference
Boer, Kalahari red, Savanna Nguni, Tswana, Venda, Xhosa, Zulu and Tankwa	216	South Africa	0.35- 0.41	0.33-0.40	0.03-0.15	Mdladla <i>et al.</i> (2016)
Nubian, Desert, Taggar and Nilotic	95	Sudan	0.39-0.39	0.39-0.40	-0.01-0.01	Rahmatalla <i>et al.</i> (2017)
Gondar, Gumuz, Agew, Ambo, Kaffa,Afar, Abergelle, Woyto-Guji and Arsi-Bale	336	Ethiopia	0.35-0.38	0.37-0.38	0.00-0.34	Mekuriaw, (2016)
Binga, Chipenge, Matopo, Shurugwi, Tsholotsho	246	Zimbabwe	0.60-0.64	0.60-0.63	-0.01-0.03	Zvinorova, (2017)
Boer, Karamojong, Kigezi, Mubende, and Small East Africa	144	Uganda	0.34-0.38	0.38-0.41	-	Onzima <i>et al.</i> (2018)

N= total number of samples, H_O= observed heterozygosity, H_E= expected heterozygosity, F_{IS}= genomic Individual inbreeding coefficient

Statistical measures of genetic diversity and Population Structure

Genetic diversity and Linkage Disequilibrium

The common factor used to quantify diversity in a population is the expected heterozygosity or gene diversity, defined as the likelihood that two alleles chosen by chance from the population are not the same (Nei, 1977; Toro & Caballero, 2005). The heterogeneity measurements range from 0 to 1, with the higher values (H_e>0) indicating greater genetic diversity and low values (H_e= 0) implying high selection pressure and inbreeding within a population (Mburu & Hanotte, 2005). Genetic diversity within and among populations can further be partitioned using analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992). The values of AMOVA range from 0 (indicating no differentiation between the overall population and its subpopulation) to 1 (showing differentiation between the populations).

Measurement of linkage disequilibrium (LD) can also be used to quantify genetic diversity within a population. LD is defined as the non-random relationship of alleles at two or more loci. This can be due to demographic factors like migration, selection and genetic drift in populations (Slatkin, 2008). Two statistical parameters are commonly used to measure LD, namely D' and r^2 . Measurements of LD based on D' statistics often require large data as it gets inflated with small data sets and when one allele is rare (Lee *et al.*, 2011). Both extend from zero (no disequilibrium) to one (complete disequilibrium), but their comprehension is not the same. For biallelic markers, D' is equal to 1 if one or more of the four probable haplotypes is not present, and less than 1 if all probable haplotypes are available (Pritchard & Przeworski, 2001). The r^2 is the squared association between alleles at two loci and is at complete disequilibrium if only two haplotypes are found within a population (Khatkar *et al.*, 2008). The formula for D' and r^2 can be expressed as;

$$r^2 = D^2 \div f(A_i)f(B_i)f(A_j)f(B_j) \text{ where;}$$

$f(A_i)f(B_i)f(A_j)$ and $f(B_j)$ are observed frequencies of alleles A_i , B_i , A_j and B_j respectively. i and j are markers. (The frequencies of allele A or B at the i or/and j markers)

$$D' = N/N-1[4N_{AABB}+2(N_{AABb}+N_{AaBB})+N_{AaBb}/2N-2 \times f(A) \times f(B)] \text{ where;}$$

N = total number of animals, N_{AABB} , N_{AABb} , N_{AaBB} and N_{AaBb} are the matching number of individuals in each genotyping group (VanLiere and Rosenberg, 2008).

Inbreeding and Effective population size

Inbreeding is another factor used for estimating genetic diversity of a population and may be defined as mating of related animals (Falconer, 1996). In a communal set up where random mating is a common phenomenon there is a possibility of closely related parents mating which can be harmful when there is an increase in deleterious alleles (Hedrick, 2013). Inbreeding of an individual is measured with the inbreeding coefficient (F_{IS}), where it is the deviation of the observed heterozygosity in an individual relative to the heterozygosity expected under random mating i.e. $F_{IS} = 1-H_o/H_e$ (Keller & Waller, 2002). When $F_{IS} > 0$ it signifies more inbreeding than is expected at random (loss of genetic diversity), whereas $F_{IS} < 0$ indicates that inbreeding occurred less often than would be expected at random (Keller & Waller, 2002).

Effective population size (N_e) is among the most important parameters in population genetics and management of animal genetic resources. It is used to assess genetic erosion (loss of genetic diversity) and assist in explaining evolution of the studied population (Tenesa *et al.*, 2007). Estimates of N_e can be approached in three ways; demographic, pedigree and marker-based approaches. Demographic approach depends on demographic narratives such as census, age and sex ratio whilst the pedigree method relies on pedigree data (Nomura *et al.*, 2001). Marker-based approach includes calculation of

N_e from LD between syntenic markers in the genome (Wang, 2005). This has been achieved through the use of molecular technologies such as dense SNP chips, which have greatly improved marker density and overcome certain limitations in the models (Corbin *et al.*, 2012). Hayes (2003) combined with Sved (1971) original formulas have become the common formulas for estimating past and present N_e in livestock populations (Qanbari *et al.*, 2010; Corbin *et al.*, 2012).

The formula is described as $N_e = ((1/E[r^2]) - 1) * (1/4c)$,

where c is the average genetic distance in Morgans estimated for each chromosome in the LD analysis and $E[r^2]$ is the expected r^2 at distance c calculated as $E(r^2) = 1 / (1 + 4N_e c)$ (Sved, 1971).

Time is in generations expressed as $T = 1/2c$ (Hayes *et al.*, 2003).

Population structure

Analyses on population structure are useful for effective management and conservation strategies as insight can be obtained on population differentiation and population dynamics such as admixture within and between populations (Patterson *et al.*, 2006). The most common approaches for illustrating population structure are principal component analysis (PCA) (Patterson *et al.*, 2006) and model-based clustering approach like STRUCTURE (Pritchard *et al.*, 2000) and ADMIXTURE (Alexander *et al.*, 2009).

PCA is a multivariate analysis that can be used to detect crossbreeding or introgression of a gene pool in the population, and historical events that happened and shaped the population during domestication and breed formation (Abdi & Williams, 2010). It entails an arithmetical formula that converts a number of probably associated variables into a smaller number of unassociated variables called principal components, which in practice is the variation between populations (Suhr, 2005). The first principal component explains as much of the variability in the whole data as possible, and subsequent component explains the remaining variability (Suhr, 2005). The principal components are acquired from the genomic relationship matrix (G) as described by VanRaden (2008);

$$G = (M - 2P)(M - 2P)' \div 2\sum p_i(1 - p_i)$$

where; M is a matrix of counts of the alleles "A" (with dimensions equal to the number of animals by number of SNP), p_i is the frequency of allele "A" of the i th SNP and P is a matrix (with dimensions equal to the number of animals by number of SNP) with each row containing the p_i values.

Admixture among populations is the basic process that forms genetic variation. The analysis usually gives insight about evolutionary history of breeds and provides an opportunity to deduce the most possible number of ancestral populations of livestock (Patterson *et al.*, 2012). ADMIXTURE software employs a maximum likelihood model based on grouping algorithm that recognizes subpopulations

that have different allele frequencies and assigns them into K predefined clusters (Alexander *et al.*, 2009). However, the option value for K is a challenging statistical problem, as it has to be directed by certain information of population history (Alexander *et al.*, 2009).

The STRUCTURE analysis program also exploits allele frequency information to distinguish parental populations, detect and allocate individuals to their populations and estimate admixed individuals (Pritchard *et al.*, 2000). This technique uses a model-based clustering method that employs a Markov Chain to estimate the posterior distribution (\hat{q}) of each individual's admixture co-efficient. The average of this distribution signifies an amount of individual genes that are acquired from one of the assumed parental populations (Kumar *et al.*, 2003).

Conclusions

Goats were domesticated around 11000 years ago from the bezoar (*Capra aegagrus*). Indigenous goats possess adaptive characteristics important to subtropical climatic and environmental conditions. These adaptive features are important and need to be conserved for current and future utilization, for breed development programmes and for general improvement of livelihoods. Characterisation of indigenous goats will provide knowledge and give clear perspective on the population structure and genetic diversity that will assist in decision making of future breeding programmes.

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CHAPTER 3

A survey analysis of indigenous goat production in communal farming systems of Botswana.

P.I. Monau^{ab*}, C. Visser^a, S.J.Nsoso^b & E. Van Marle-Köster^a

^aDepartment of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa

^bDepartment of Animal Science, Botswana University of Agriculture and Natural Resources, Private bag 0027, Gaborone, Botswana.

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A survey analysis of indigenous goat production in communal farming systems of Botswana

P.I. Monau^{ab*}, C. Visser^a, S.J.Nsoso^b & E. Van Marle-Köster^a

^aDepartment of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa

^bDepartment of Animal Science, Botswana University of Agriculture and Natural Resources, Private bag 0027, Gaborone, Botswana.

*Corresponding Author: E-mail address: phetogomonau@gmail.com

Abstract

A total of 153 communal farmers in four agro-ecological regions of Botswana were interviewed using a structured questionnaire. The aims of the survey were to characterise existing communal goat production systems, evaluate the importance of goats to farmers and identify breeding practices and constraints encountered in goat production in Botswana. Data was collected on socio-economic parameters, general and breeding management practices and major constraints limiting goat production in Botswana. All respondents were small-scale communal farmers with 63% respondents practising mixed crop-livestock farming and 37% keeping livestock as their primary activity. The majority (33%) of respondents were older than 60 years. Over 80% of the farmers kept goats for cash required for tuition, school uniforms and household commodities as well as re-stocking of animals. Most farmers (62%) kept indigenous crossed genotypes. Generally uncontrolled mating was practised with the majority of farmers (41%) using on-farm reared bucks for more than two years of breeding and communal bucks (36%) as an alternative. The major constraints limiting goat productivity in communal areas included uncontrolled breeding, predators, theft and diseases. Issues raised by farmers should be considered in designing and implementing effective breeding programs for goats to improve their overall productivity and contribution to poverty alleviation in these communities.

Keywords: Botswana, Communal, Production system, Smallholders

Introduction

Indigenous goats are important to resource-poor rural farmers as they contribute to food security and poverty alleviation. They are known for their ability to adapt to a variety of climatic conditions and reproduce under low input systems (Lebbie, 2004). Indigenous goats represent over 95% of small ruminant populations in Africa of which approximately 90% are owned by rural households (Chiejina and Behnke, 2011). Despite this, indigenous goats have often received less attention from researchers, funding institutions, veterinarians and other stakeholders when compared to other livestock species (de Vries, 2008), resulting in limited genetic improvement and overall productivity. Some genetic improvement programmes for indigenous goats in tropical countries have collapsed due to the incompatibility of breeding objectives, failure to capture cultural (intangible) values and the prevailing management method of low input production system (Tibbo *et al.*, 2006), such as the dairy

development project in Ethiopia where local breeds were crossed with exotic Anglo-Nubian goats to improve milk yield. The crossbreeds however, failed to outperform the indigenous goats under similar management systems (Ayalew *et al.*, 2003).

Botswana has approximately 1.6 million goats (Botswana Statistics, 2015) that contribute significantly to the livelihoods of rural farmers under communal production systems. Communal production systems in Botswana, as in other developing countries, are characterised by low input and productivity levels, lack of infrastructure and no properly defined breeding strategies (Kosgey, 2004). The majority of goats in Botswana belong to the indigenous Tswana breed, characterised as multi-coloured, medium sized goats with long lopping ears, short coarse hair and are often bearded and horned (Nsoso *et al.*, 2004b). Although limited research has been conducted on the breed and production systems, it has been reported to have valuable traits such as tolerance towards diseases, drought and heat (Nsoso *et al.*, 2004b). Evaluation of goat production systems through identification and prioritization of constraints is a prerequisite for planning and improving production. This study aimed to characterise existing goat production systems with regard to breeding practices and constraints encountered in communal goat production in four agro-ecological regions of Botswana.

Materials and methods

A structured questionnaire was used to investigate the production and breeding systems of indigenous goats in Botswana. The questionnaire was a slightly modified version of those designed for the livestock breed survey in Southern Africa (Rowland *et al.*, 2003). The questionnaire included socio-economic parameters (e.g. sex, age, education level, economic value of goats and production constraints), breeding management and goat production systems of each participant. Participants were asked to rank their major sources of income, reasons for keeping goats, buck selection criteria and major constraints (ranging from 1 to 3, where 1=most important and 3=least important). Information was obtained through an oral interview for which individual consent was given by the farmers.

Sampling strategy

The country was divided into four regions: Southern region which is mainly hard-veldt, the Central region that is hard-veldt with *Colospospermum mophane* trees being dominant, the Northwest region which is sandveldt with thick forest, lush green plains and semi-arid shrub savanna trees and the Ghanzi region which is sandveldt with low trees and shrub savanna trees. Random sampling was used to select districts, villages and households that kept goats in each region. In the Northwest and Ghanzi regions only two districts were surveyed per region because of inaccessibility due to poor terrain. The survey was conducted from December 2015 to March 2016.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS, 2007). Chi-square tests (χ^2) were used to assess the statistical differences, with $P < 0.05$ as significance level. An index described by Kosgey (2004) was used to calculate rankings, and indicated the criterion's relative importance to the household.

Results

A total of 153 households were interviewed in four agro-ecological regions of Botswana namely: Southern (n=43), Central (n=54), Northwest (n=36) and Ghanzi (n=20). There was no significant ($P > 0.05$) difference in sex of head of households across the regions. The Central and Ghanzi regions had a significantly ($P < 0.05$) higher number of respondents older than 60 and between 46 to 60 years of age, respectively. The most common level of education was primary education in almost all the regions except in the Northwest region where most (42%) respondents had a secondary level of education. The Southern region had significantly more (14%) respondents with tertiary education than the other regions. The majority of respondents in all regions were small-scale communal farmers practising mixed crop-livestock farming (Table 3.1). The major crops grown included maize, sorghum, millet and cowpea.

Table 3.1: Socio-economic characteristics of households (%) keeping goats in the four regions of Botswana included in this study.

Descriptors	Southern region	Central region	Northwes t region	Ghanz i region	Overall total	X ² P value
Gender						
Male	65	50	47	45	53	
Female	34	50	52	55	47	0.29
Age (years)						
≤ 30	12 ^a	17 ^a	14 ^a	0.0 ^b	12	
31-45	21 ^a	26 ^a	31 ^a	45 ^a	28	
46-60	33 ^b	13 ^c	33 ^b	40 ^a	27	0.03
over 60	35 ^b	44 ^a	22 ^b	15 ^b	33	
Education						
None	16 ^a	28 ^a	22 ^a	35 ^a	24	
Primary	49 ^a	43 ^a	36 ^a	40 ^a	42	
Secondary	21 ^b	28 ^b	42 ^a	25 ^b	29	0.04
Tertiary	14 ^a	2 ^b	0 ^b	0 ^b	5	
Production system						
Livestock only	42 ^b	15 ^a	3 ^c	20 ^a	20	
Crop-Livestock	58 ^b	85 ^a	97 ^c	80 ^a	80	0.00

^{abc} frequencies of households within a row with different superscript are significant different ($P < 0.05$)

Cattle, goats, sheep, donkeys and poultry were the major livestock species kept in the study areas. On average, 24.3 ± 1.4 goats were kept per household with bigger average herd sizes of cattle and goats observed in the Ghanzi region (Table 3.2).

Table 3.2: Average number of livestock kept per household in the four surveyed regions of Botswana

Species	Southern	Central	Northwest	Ghanzi	Overall
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Goats	22.5 ± 2.4	22.3 ± 1.9	25.5 ± 3.7	31.0 ± 4.6	24.3 ± 1.4
Cattle	5.2 ± 1.6^a	4.0 ± 1.1^a	11.5 ± 3.3^{ab}	18.8 ± 6.1^b	8.0 ± 1.3
Sheep	4.2 ± 1.3	2.9 ± 1.3	0.2 ± 0.2	3.3 ± 3.3	2.7 ± 0.7
Chicken	7.6 ± 1.6	6.5 ± 1.0	4.2 ± 1.1	6.0 ± 1.1	6.2 ± 0.7
Donkeys	0.2 ± 0.1^a	2.0 ± 0.5^b	0.7 ± 0.3^a	1.3 ± 0.4^{ab}	1.1 ± 0.2

^{a,b} means on the same row with different superscripts are significantly different ($P < 0.05$). SE= Standard Error

Farmers reported and ranked varying major sources of income amongst the regions, ranging from piece jobs (0.37) in the Southern region to the sale of crops (0.37) in the central region and sale of livestock in the Northwest (0.39) and Ghanzi (0.43) regions. The main reason for keeping goats was as a source of income (mainly used for school fees and school uniforms, household commodities and re-stocking of animals). Goats were also kept as a source of meat for home consumption (highly ranked after cash), especially in the Southern region. Keeping goats for breeding was ranked least by respondents across all regions (Table 3.3).

Table 3.3: Frequency¹ (%) of source of income, reasons for keeping goats and the ranking of these descriptors as described by respondents in four surveyed regions of Botswana

Descriptors	Southern	Rank	Central	Rank	Northwest	Rank	Ghanzi	Rank
	n region	index	region	index	t region	index	region	index
Source of								
Income								
Livestock	30	0.22	41	0.19	86	0.39	75	0.43
Product								
Crops	16	0.13	66	0.37	61	0.34	40	0.20
Piece Jobs	49	0.37	46	0.27	39	0.17	45	0.23
Salary/wages	33	0.28	32	0.18	20	0.10	20	0.14
Purpose								
Cash	77	0.33	81	0.30	89	0.34	90	0.40
Meat	81	0.34	57	0.20	39	0.20	55	0.23
Milk	61	0.23	53	0.18	25	0.08	30	0.08
Insurance	12	0.04	51	0.17	69	0.26	40	0.16
Investment	5	0.01	11	0.06	17	0.05	25	0.10
Culture	7	0.02	9	0.03	11	0.04	5	0.02
Ceremony	7	0.02	6	0.02	8	0.03	5	0.02
Breeding	2	0.00	8	0.03	6	0.01	0	0.00

¹Multiple answers were possible, thus frequency (%) will add to more than 100

Most of the respondents across regions kept crossbred genotypes. Farmers in almost all the regions preferred Boer goat bucks, except in the Central region where indigenous Tswana bucks were preferred, and a significant number of indigenous Tswana goats were kept due to less susceptibility to diseases. In the Ghanzi region a significant ($P < 0.05$) number of respondents prefer exotic breeds, such as Boer goat and Kalahari Red due to less prevalence of tick infestation in the area. The majority (73%) of households across regions practised uncontrolled mating, with the highest ($P < 0.05$) incidence in the Northwest region. Farmers generally reared their own breeding bucks for 2 to 4 years and alternatively, communal bucks were used (Table 3.4).

Table 3.4: Frequency (%) of breed kept, mating system, source of buck and duration of buck for breeding in the four surveyed regions of Botswana.

Descriptors	Southern region	Central region	Northwest region	Ghanzi region	Overall Total	X² P value
Breed kept						0.00
Indigenous	16 ^b	39 ^a	31 ^a	10 ^b	27	
Pure exotic	2 ^c	4 ^c	17 ^b	40 ^a	11	
Indigenous*exotic crosses	81 ^a	57 ^b	53 ^b	50 ^b	62	
Mating						0.01
Uncontrolled	56 ^c	76 ^b	89 ^a	70 ^b	73	
Group mating	5 ^b	0 ^b	0 ^b	10 ^a	3	
Controlled	40 ^a	24 ^b	11 ^c	20 ^b	25	
Source of buck						0.78
Borrowed	7	4	3	10	5	
Hired	7	4	3	0	4	
Bought	16	11	8	20	13	
Own flock	40	41	41	45	41	
Communal area buck	30	41	41	25	36	
Other	0	0	3	0	1	
Breeding buck						0.18
Boer	48	36	43	57	44	
Tswana	16	45	29	7	27	
Boer x Tswana	36	16	24	29	25	
Kalahari x Tswana	0	3	0	7	2	
Saanen	0	0	5	0	1	
Years of buck for breeding						0.28
1-2 years	32	18	10	21	21	
2-4 years	36	39	62	29	42	
≥ 5 years	32	42	29	50	33	

^{abc} frequencies of mating system within a row with different superscript are significant different ($P < 0.05$)

Selection criteria for breeding bucks was mostly based on body conformation (0.5) and body size (0.3) other than performance history (0.1), availability (0.0) and colour (0.1) (Table 3.5).

Table 3.5: Ranking of selection criteria of buck as reported by households (%) in the four surveyed regions of Botswana.

Reasons for choice of Buck	Southern region	Rank Index	Central region	Rank Index	Northwest region	Rank Index	Ghanzi region	Rank Index
Body conformation	58	0.5	56	0.5	47	0.5	70	0.4
Body size	56	0.3	50	0.3	42	0.3	70	0.3
Colour	19	0.1	19	0.2	25	0.2	10	0.1
Performance	19	0.1	4	0.0	11	0.0	15	0.1
Availability	9	0.0	4	0.0	8	0.0	10	0.1

Multiple answers were possible, thus frequency (%) will add to more than 100

The major constraints limiting goat production are shown in Figure 3.1. Predation (0.28) was highly ranked by participants as one of the main constraint affecting goat production in Botswana. Stock theft (0.24), diseases (0.23) and drought (0.13) were other major constraints reported by farmers across the regions.

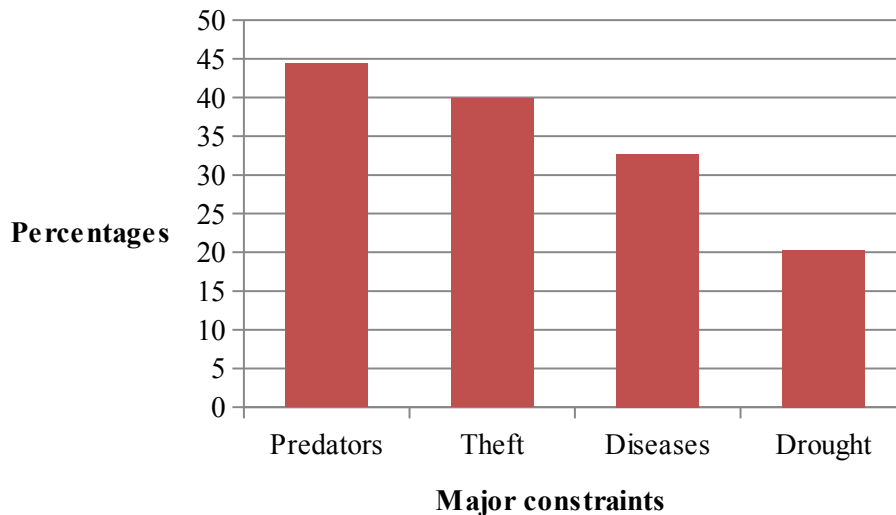


Figure 3.1 The major constraints limiting goat production in the four surveyed regions of Botswana

Discussion

The design of sustainable genetic improvement programmes requires an understanding of the production systems and constraints affecting them. Demographic characteristics of the current study have shown reasonably good participation of women in goat production, which indicates an increase in female economic autonomy and bargaining power within the households (Dossa *et al.*, 2008). Most of the households attained relatively low levels of education which could have an impact on production methods, management ability, record keeping and accessing of market information (Kosgey *et al.*, 2006). Training of farmers will therefore, empower them and enhance the potential success of breeding programs which depend profoundly on record keeping (Kosgey and Okeyo, 2007).

The low participation of youth observed in this study was also reported by Nsoso *et al.* (2004a) in a survey on goat production in the Kweneng district of Botswana. The youth generally perceive agriculture as a non-viable sector of employment (FAO, 2012), and are discouraged by constraints such as limited capital, land, water, access to markets and inadequate involvement in policy dialogue (FAO, 2014). This is unfortunate as they are the future generation of farmers that should ensure the future of the industry. As a way of encouraging youth participation in farming, the Botswana government grants loans of 5% interest with 24 months' grace period as start-up capital to youth (18 to 40 years) for any agricultural project (FANRPAN, 2007). However, most of these projects collapse as soon as government support is withdrawn probably due to lack of marketing opportunities and inappropriate breeding objectives.

Most respondents in all regions were not formally employed and depended on a mixed crop and livestock production system for financial income and household food security. This dependency of rural livelihoods on crop and livestock production is a common phenomenon in developing countries and is also seen as an opportunity for efficient use of resources (Kosgey and Okeyo, 2007). The overall flock sizes were similar to those reported by Dube *et al.*, (2015) in the Eastern Cape (South Africa) but inconsistent with Homann *et al.*, (2007) who reported lower flock size of 8 per household in Zimbabwe. The large flock sizes and record keeping are important for development of breeding programs especially for selection intensity and genetic gain (Shumbusho *et al.*, 2013). However, lack of sustainable performance recording in livestock has been a problem in most developing countries (Kogey *et al.*, 2006)

Variation of source of income across the regions was influenced by several factors such as climate, soil conditions and proximity to the urban areas. For instance, in the Ghanzi and Northwest regions major source of income was influenced by poor crop production due to restricted climatic conditions. In these regions livestock production may present a viable alternative. The good soil and moderate climatic conditions in the Central region (Mogotsi *et al.*, 2013) supports the high frequency of crop production as a source of income. The respondents in the Southern region preferred piece jobs in urban or peri-urban areas for day-to-day cash flow. In all regions goats were kept and used as an additional source of food and potential income. The results indicate the multiple needs of the family, dependant on crops and livestock, especially goats, for maintaining their livelihoods. The importance of goats to the households clearly requires a strategy for genetic improvement and sustainable utilization.

The observed high frequency of households keeping crossbreds is in contrast with the findings of Kosgey *et al.* (2008) where farmers in Kenya predominantly kept indigenous goats. The initial purpose of crossbreeding was to improve the growth of indigenous Tswana goats in order to obtain higher

revenues. However, this was performed indiscriminately without the desired improvement in goat performance or farmers' livelihoods. Similarly, selection criteria for bucks were mostly based on growth traits that will ultimately increase income. The desire for larger animals has also been expressed in pastoralists' flocks in Kenya (Kosgey *et al.*, 2008). The constitution of adaptive and growth traits will be important in designing breeding programs for goat production in communal areas of Botswana.

The observed uncontrolled random mating across the regions in this study was due to unfenced communal grazing lands, an insufficient number of breeding bucks and a lack of skills and understanding of the adverse effect of inbreeding. Gwaze *et al.* (2008) and Kosgey *et al.* (2008) have reported similar practises. Uncontrolled mating leads to an absence of fixed kidding seasons and inbreeding (Jimmy *et al.*, 2010). Parturition throughout the year requires high levels of managerial skills, such as dosing against antihelmentics and weaning, which is generally lacking in communal production systems. Inbreeding is exacerbated by small flock sizes, a lack of animal recording and the long periods that bucks stay in the flocks before they are culled. Subsequent inbreeding depression leads to decreased fitness (e.g. low reproduction rates and high mortality) as well as small body sizes and poor growth rates (Jimmy *et al.*, 2010).

Communal fields were not fenced and goats were not herded which predisposed them to predators and theft, which is consistent with constraints reported elsewhere in Africa (Gwaze *et al.*, 2009, Fikru and Gebeyew, 2015). Infectious diseases and parasites were also a serious constraint to communal goat production in this study. This is an endemic situation in many regions of Southern Africa (Githiori *et al.*, 2006; Gwaze *et al.*, 2008), where livestock are usually reared extensively, and it is difficult to implement control measures. Low management levels such as drinking dirty water from rivers and poor housing also predisposes animals to diseases (Peacock, 2005). Most animals in this study were kept in a kraal with piled up manure and little protection against extreme weather conditions. Although indigenous goat breeds exhibit higher tolerance to local diseases and gastrointestinal parasite infestation (Bishop and Morris, 2007), veterinary management remains imperative to improve overall productivity and animal welfare.

The revealed challenges are essential in genetic improvement and development of sustainable breeding programs. There is neither a systematic goat breeding program nor a goat breeding policy in Botswana. An improvement program launched by the Botswana government aims to place goats with rural households to improve food security and general livelihoods (MoA, 2006). The success of this program will depend on adequate knowledge transfer and proper breeding objectives at national and regional level with relevant stakeholders to improve goat productivity. The above mentioned

constraints can be mitigated by designing a community breeding program suitable for traditional low input livestock production systems.

Conclusions

Goats contribute significantly to the livelihoods of smallholders, and are kept in mixed crop-livestock systems in the communal areas of Botswana. Several constraints hinder goat productivity in Botswana and these should be considered when designing and implementing genetic improvement programmes. It is paramount to look at the production system holistically and involve all stakeholders in designing and implementing effective breeding programmes. This will assist in using scarce resources efficiently and designing appropriate technologies which are compatible with the production system.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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CHAPTER 4

Phenotypic and genetic characterisation of indigenous Tswana goats

P.I. Monau^{1,2}, C. Visser¹, S.J.Nsoso² & E. van Marle-Köster^{1#}

¹Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa

²Department of Animal Science, Botswana University of Agriculture and Natural Resources, Private bag 0027, Gaborone, Botswana.

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P.I. Monau^{1,2}, C. Visser¹, S.J.Nsoso² & E. van Marle-Köster^{1#}

¹Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa

²Department of Animal Science, Botswana University of Agriculture and Natural Resources, Private bag 0027, Gaborone, Botswana.

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Abstract

Tswana goats that were kept in communal systems in three agro-ecological regions in Botswana were characterized according to phenotypic measurements and genotypic data. Objective measurements for 123 goats included bodyweight (BW), body length (BL), heart girth (HG), height at withers (HW), and tail length (TL), while qualitative traits included coat colour and presence or absence of horns and beards. Age was estimated based on dentition. Hair samples were collected from 48 of the phenotyped animals in the largest region (central region) and genotyped with the Illumina Goat50K SNP chip. Mixed coat colour was predominant and across regions 95% of the goats were horned and bearded. Goats in the northwest region had the lowest BW and significantly higher HG values in all age groups compared with other regions. Goats over four years old in the central and northwest regions were significantly longer in body compared with the ones from the southern region. The average expected heterozygosity and inbreeding coefficient were 0.423 ± 0.03 and 0.009 ± 0.05 , respectively. Principal component analysis clustered most animals, with a few outliers. The effective population size has decreased over time and at 13 generations ago was estimated at 266. There were high genetic and phenotypic variations in the indigenous Tswana goats, which should be exploited to increase performance through within-breed selection and structured crossbreeding.

Keywords: Botswana, genomic diversity, morphometric, population size, Single nucleotide polymorphism.

[#]Corresponding author: Este.vanMarle-Koster@up.ac.za

Introduction

Characterization of indigenous livestock species is the key to development of proper strategies for long-term maintenance and use of genetic variation, and for guidance in decisions about future utilization and conservation strategies (Msanga *et al.*, 2012). In southern Africa, limited studies have been conducted to characterize indigenous livestock, particularly goats. These include Mozambican goats (Garrine, 2007), Tanzanian goats (Madubi *et al.*, 2000; Nguluma *et al.*, 2018), South African goats (Pieters *et al.*, 2009; Mdladla *et al.*, 2016), and Namibian goats (Els *et al.*, 2004). However, information on the goat characterization in the region is still scarce, as several indigenous populations are not managed through herd book registration (Van Marle-Köster *et al.*, 2015). Although crossbreeding is important for hybrid vigour and breed complementarity, if it is indiscriminate it poses a threat to most indigenous goat populations as it results in a decline in the adapted traits, ultimately reducing the efforts at improving food security in rural areas (Rischkowsky, *et al.*, 2007).

In Botswana, there are approximately 1.6 million goats. The main indigenous breed is the Tswana goat, which constitutes 71% of the national goat population (Botswana Agricultural Statistics, 2013). This breed is found in various geographical regions of the country under low-input management systems and contributes significantly to the livelihoods of resource-poor farmers as a source of protein and income (Monau *et al.*, 2017). The Tswana goat is known for its unique adaptive traits such as heat and drought tolerance, and lower disease susceptibility, which make it ideal for production under stressful tropical environments (Katangole *et al.*, 1996; Nsoso *et al.*, 2004). However, it has received little attention in research projects. The last phenotypic characterization studies on Tswana goats were undertaken more than a decade ago (Nsoso *et al.*, 2004), and may not reflect the current situation, because of changes in population and in production systems (Solkner *et al.*, 1998). The only previous effort to characterize indigenous Tswana goats at genetic level was done at a research station, using 12 microsatellite markers on a very small population that was under selection (Maletsanake *et al.*, 2013). This population did not represent the variation that occurs in the country, and failed to show inherent genetic variation of the Tswana goat population.

Although the advantages of microsatellites have been well documented (Singh *et al.*, 2014), genome-wide single nucleotide polymorphism (SNP) markers provide new possibilities for genetic characterization and biodiversity studies (Blasco & Toro, 2014). Several SNP assays have been used in the analyses of population diversity and structure of many livestock species (Lin *et al.*, 2010; Kanyile *et al.*, 2015; Makina *et al.*, 2015; Lashmare *et al.*, 2016; Mdladla *et al.*, 2016). The commercial Goat50K SNP panel, which was developed in 2012 (Tosser-Klopp *et al.*, 2014), offers an opportunity for genetic characterization of goats.

The purposes of this study were to phenotypically characterize Tswana goats in three agro-ecological regions of Botswana and to assess the genetic diversity and population structure of this genetic resource in the central region using the Goat50K SNP panel.

Materials and method

The use of animals complied with the guidelines approved by the Ethics Committee of Faculty of Natural and Agricultural Sciences at the University of Pretoria (ECO42-15).

The study was carried out in Botswana from December 2015 to March 2016. The climate of the country is mainly semi-arid. Rainfall occurs during the summer months of October to April and is low, unreliable, unevenly distributed, and highly variable from year to year (Makhabu *et al.*, 2002). The highest average temperatures generally occur from October to April and the lowest from May to August. Drought is a recurrent phenomenon, and all rivers in the country are seasonal, except for the Okavango and Linyanti/Chobe rivers in the northwest (Mogotsi *et al.*, 2013). Geographically, soil varies from one region to another, with about two thirds of the country being covered in infertile sandy soils (red and grey desert soils) (Mogotsi *et al.*, 2011). Four agro-ecological regions are distinguished in Botswana, with the southern region being classified as hardveldt, the central region as hardveldt with the dominance of woodland *Collospermum mophane* trees, the northwest as sandveldt with thick forest, lush green plains and semi-arid shrub savanna trees, and the Ghanzi as sand veldt with shrub savanna trees.

Random sampling was performed to select representative districts, villages and farms, based on knowledge of livestock (particularly goats) from livestock extension officers in each region. In each village, four to five farms were randomly selected and one to five unrelated Tswana goats per farm were measured. To avoid sampling related individuals, farmers stated the origin and familial relationships of individual animals.

Animals were grouped into five age categories based on dentition, namely no pairs of permanent incisors (<14 months), one pair of permanent incisors (15–23 months), two pairs of permanent incisors (24–35 months), three pairs of incisors (36–48 months), and four pairs of incisors (over 48 months), according to Pace & Wakeman (2003). Bodyweight (BW) was measured using a hanging scale. Body length, HG, HW, and TL were measured with a tailor's tape following the standard procedure reported by FAO (2012). Morphological descriptions such as coat colour and presence or absence of horns and beard were also recorded. One hundred and twenty-three goats were measured in total, of which 47, 54 and 22 were located in the southern, central and northwest regions, respectively. No Tswana goats were observed in the Ghanzi region. All goats were kept under extensive systems in communal areas and were not selected for production traits.

Hair samples were collected from forty-eight of the animals in the central region, as this is the largest region (142,302 km²). Hair samples were collected by plucking 50 to 100 hairs from the tail of each animal, ensuring intact follicles. The hair samples were kept in labelled envelopes and transported to the University of Pretoria laboratory and then shipped to Animal Genetic Laboratory in France, Labogena DNA platform (Domaine de Vilvert, CS 80009, 78353 JouyenJosascedex), for DNA extraction and genotyping.

Procedure frequency was used to analyse qualitative traits and general linear model was used to analyse quantitative traits in Statistical Analysis System (SAS)(SAS Institute, 2009). Owing to low numbers of animals at 0–14 months and 15–23 months, only three age categories were used, that is, 24–35 months, 36–48 months, and above 48 months. Analysis was also confined to females owing to the small number of males (castrates and bucks) that were kept. Fixed effects were considered to significantly affect performance when $P < 0.05$. This statistical model was implemented to analyse BW and body measurements:

$$Y_{ijk} = \mu + \text{age}_i + \text{region}_j + e_{ijk}$$

Where Y_{ijk} = observation on body measurement

μ = population mean

Age_i = age ($i = 1, 2, 3$, age at measurements)

Region_j = region ($k = 1, 2, 3$)

e_{ijk} = normal distributed random error

Forty-eight animals from the central region were genotyped using the Illumina Goat50K SNP Beadchip, which contains 53,347 SNPs. Only autosomal SNPs were considered, while SNPs on the sex chromosomes and SNPs with unmapped locations to the latest reference assembly of the goat genome were excluded from the analysis. Plink version 1.07 software (Purcell *et al.*, 2007) was used for analysis. Quality control was performed with these standard thresholds: individual call rate lower than 97%, SNP call rate less than 97%, SNPs with MAF below 0.05, and SNPs that deviated significantly from Hardy-Weinberg equilibrium ($P < 0.001$). After quality control, genetic diversity parameters (observed and expected heterozygosity) and average individual inbreeding coefficient across autosomes were calculated using Plink software (Purcell *et al.*, 2007). Principal component analysis (PCA) was performed using genome-wide complex trait analysis (GCTA version 1.24; Yang *et al.*, 2011). ADMIXTURE version 1.23 (Alexander *et al.*, 2009) was used to investigate population differentiation based on SNP genotype data. A cross-validation (CV) procedure was performed on 48 animals to choose for optimal K-value with the lowest CV error values.

After quality control, pair-wise linkage disequilibrium (LD) was assessed through the correlation coefficient (r^2) using Plink software (Purcell *et al.*, 2007). The command ‘-r2 -ld-window-kb 2000 -ld-window-r2 0’ was used to calculate the association among SNP pairs up to a distance of 2000 kb. To inspect the LD decay with physical distance, SNP pairs were sorted into bins based on pair-wise marker distance and the average of each bin was calculated. These bins were defined, namely 0–10, 10–20, 20–40, 40–60, 60–80, 80–100, 100–200, 200–500, 500–1000, and 1000–2000kb.

Effective population size (N_e) was determined based on r^2 values at various distances and assuming a model without mutation, as described by Corbin *et al.* (2010) using SNep version 1.1 (Barbato *et al.*, 2015). Minimum and maximum inter-SNP distances of 0 and 1000Mb, respectively,

were used with 30 distance bins of 50kb each. N_e estimates were subsequently calculated from r^2 values obtained for the average distance of each bin.

Results

Based on the surveyed regions, adult goats dominated the flock structure across regions with 29% in the 24–35 month age group, 42% in 36–48 months and 23% over four years old. The young and grower age groups of 0–14 months and 15–23 months accounted for only 2% and 4% of the flocks, respectively. However, the numbers of goats older than 48 months in the southern and northwest regions were significantly ($P < 0.05$) less compared with central region. Across all regions, Tswana female goats were predominant (92%) (Table 4. 1).

Table 4.1 Flock structure of indigenous Tswana goats in surveyed regions of Botswana

Parameters	Regions			Overall	Overall
	Southern	Central	Northwest	Total	Percentage (%)
Age					
0–14 months	2	0	0	2	2
15–23 months	2	3	0	5	4
24–35 months	13	16	7	36	29
36–48 months	26	17	9	52	42
Over 48 months	4 ^a	18 ^c	6 ^b	28	23
Sex					
Intact males	0	1	1	2	2
Females	43	50	20	113	92
Castrated males	4	3	1	8	6

^{abc}Different superscripts within a row differ significantly ($P < 0.05$)

Almost all goats (95%) were horned and bearded. Seven coat colours were observed across all regions, with mixed colour (28%), brown and white (23%) and black and white (23%) patterns being the most prominent. Evenly coloured goats, that are brown, black or white, were less common (Table 4.2).

Table 4.2 Qualitative traits of indigenous Tswana goats in various agro-ecological regions of Botswana

Parameters	Regions			Overall	Overall
	Southern	Central	Northwest	Total	Percentage
Coat colour					
Mixed colour	13	15	6	34	28
Brown and white	10	13	5	28	23
Black and white	12	12	4	28	23
Brown and black	7	7	2	16	13
Brown	4	3	4	11	9
Black	1	2	0	3	2
White	0	2	1	3	2
Horn					
Horned	42	53	22	117	95
Polled	5	1	0	6	5
Beard					
Presence	47	49	21	117	95
Absence	0	5	1	6	5

Morphological differences were observed between regions, with HW being significantly ($P<0.05$) lower at 24–35 months in southern region compared with the northwest. Goats in the northwest region had significantly ($P<0.05$) lower BW and higher HG values at 36–48 months and at >48 months. Body length measurement was significantly lower in the southern region compared with central region at over 48 months. The tails of goats from the central region were significantly longer than those from the southern region at over 48 months (Table 4.3). Most morphological differentiation based on region could be observed in the mature goats, older than four years.

Table 4.3 Mean (\pm SE) for bodyweight and body measurements of female Tswana goats

Age	Region	Body weight	Body length	Height at withers	Heart girth	Tail length
24–35 month s	Southern	29.27 \pm 0.8	63.31 \pm 1.3	62.08 \pm 1.1 ^a	70.85 \pm 1.3	20.39 \pm 0.7
	Central	30.27 \pm 0.7	63.00 \pm 1.2	63.73 \pm 1.0 ^{ab}	70.27 \pm 1.2	20.47 \pm 0.7
	Northwes t	28.20 \pm 1.2	64.00 \pm 2.1	66.40 \pm 1.7 ^b	74.60 \pm 2.1	20.60 \pm 1.2
36–48 month s	Southern	34.00 \pm 0.5 ^b	67.58 \pm 0.9	65.42 \pm 0.8	72.89 \pm 0.9 ^a	20.31 \pm 0.5
	Central	32.75 \pm 0.7 ^{ab}	66.65 \pm 1.2	63.81 \pm 1.0	72.69 \pm 1.2 ^a	22.06 \pm 0.7
	Northwes t	31.22 \pm 0.9 ^a	67.56 \pm 1.5	66.11 \pm 1.3	76.67 \pm 1.6 ^b	20.33 \pm 0.9
Over 48 month s	Southern	33.75 \pm 1.4 ^{ab}	64.25 \pm 2.3 ^a	65.75 \pm 2.0	70.75 \pm 2.3 ^a	19.25 \pm 1.3 ^a
	Central	34.67 \pm 0.6 ^b	71.83 \pm 1.1 ^b	65.89 \pm 0.9	76.11 \pm 1.1 ^b	22.89 \pm 0.6 ^b
	Northwes t	31.17 \pm 1.1 ^a	69.17 \pm 1.9 ^{ab}	65.83 \pm 1.6	80.50 \pm 1.9 ^c	21.00 \pm 1.1 ^{ab}

^{ab}Means with different superscripts within a column, age group and trait differ significantly ($P < 0.05$).

The average genotyping call rate across 48 animals was 99.6%. No individuals were removed. A total of 5203 SNP markers were removed during quality control, namely 2338 because of low call rate (< 0.97), 2728 owing to low MAF (< 0.05) and 137 markers that violated HWE ($P < 0.001$). This resulted in 44741 SNP markers that were retained for further analysis. The MAF of SNPs followed a uniform distribution across the chromosomes and averaged at 0.32 ± 0.13 . The average observed and expected heterozygosity was 0.419 ± 0.02 and 0.423 ± 0.03 , respectively. The average individual inbreeding coefficient was 0.009 ± 0.05 .

Breed composition was assessed for the 48 genotypes using a PCA in which most of the animals were clustered, with a few outliers. Total variation for first and second principal components was 1.9% and 1.4%, respectively (Figure 4.1). To investigate whether there was genetic differentiation, an ADMIXTURE version 1.23 (Alexander *et al.*, 2009) was performed and the lowest cross-validation error was at $K=1$.

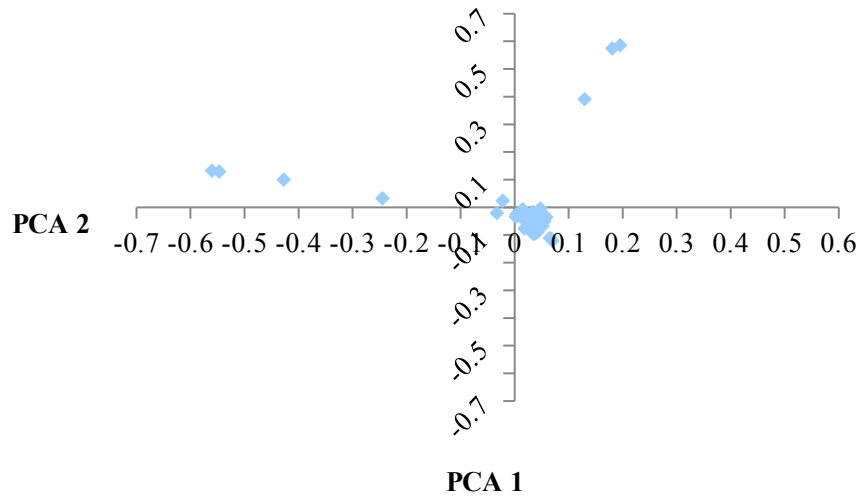


Figure 4.1 Genetic relationship among 48 Tswana goats sampled from the central region of Botswana (PCA1 vs. PCA2)

The average linkage disequilibrium (LD) values at given distance intervals for the population are displayed in Figure 4.2. The highest average r^2 of 0.44 was observed at 0–10kb. LD declined with increasing distance between SNP pairs, and the most rapid decline was seen over the first 10kb. The average r^2 for all pair-wise adjacent SNP autosomes was 0.067 ± 0.10 , with an average distance between SNP pairs of 255.8 kb.

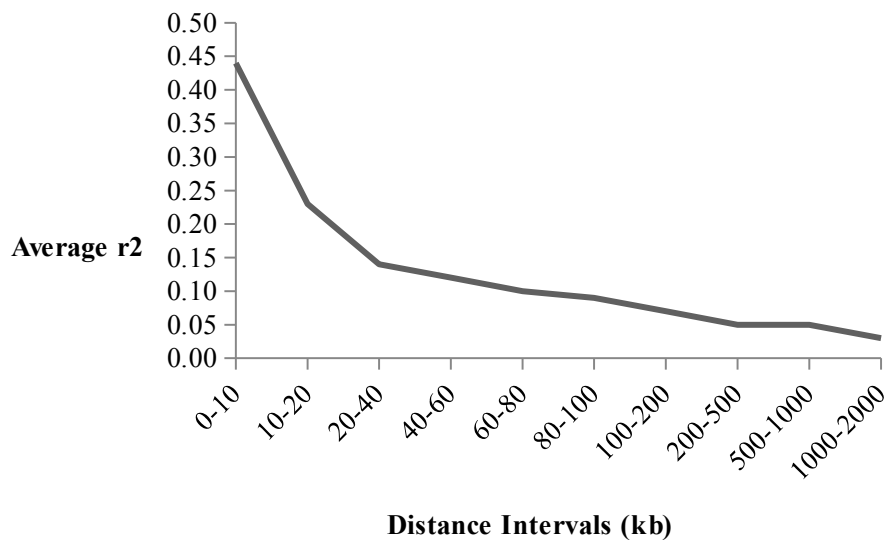


Figure 4.2 Decay of LD at given distances (kb) for Tswana goat population

A graphic representation of the effective population size (N_e) at each point from 900 to 13 generation ago is given in Figure 4.3. The results show a progressive decrease in N_e over time with an estimation of 266 animals at 13 generations ago.

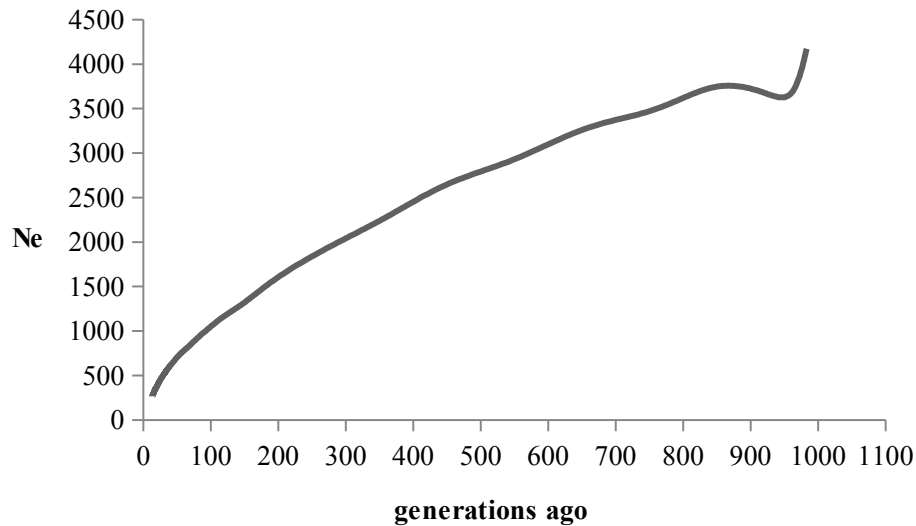


Figure 4.3 Average effective population size for central region Tswana goats, from 900 to 13 generations ago.

Discussion

Indigenous goats are valuable genetic resources, particularly for resource-poor smallholder farmers, owing to their ability to thrive in diverse geographical environments with limited inputs (Huson *et al.*, 2014). The flock structure of the indigenous population indicated that the flocks generally consist of mostly female animals. Farmers prefer to sell males for income, slaughter them for home consumption, or give them as gifts (Monau *et al.*, 2017). Mdladla *et al.* (2017) reported a similar observation in various provinces of South Africa. Additionally, farmers prefer to keep exotic bucks such as Boer goats for crossbreeding, which contributed to lower numbers of young (i.e. 0–14 and 15–23 months) indigenous goats being kept. This is in contrast with a previous study, which reported a significant number of indigenous Tswana males of 0–12 months in the flocks (Nsoso *et al.*, 2004). A decrease in the number of indigenous breeding bucks is one of the factors that threaten the existence of an indigenous breed (Mandal *et al.*, 2014). Tswana goats could be endangered or vulnerable, and it is important to strategize and conserve this breed for sustainable utilization and future breeding programmes.

Peculiar morphological characteristics of Tswana goats include a mixture of coat colour patterns and the presence of horns and beards (Katangole *et al.*, 1996). Farmers could unintentionally

be selecting towards these traits as an environmental adaptation mechanism for thermoregulation and predation. Predation is one of the major constraints affecting goat production in Botswana (Monau *et al.*, 2017), and animals use their horns for protection, while mixed coat colour patterns act as camouflage (Hagan *et al.*, 2012). Coat colour is also useful in protecting deep tissue against excess exposure to solar shortwave radiation in tropical zones (Castanheira *et al.*, 2010). The predominant mixed coat colour could be an advantage during the common seasonal temperature fluctuations in the country. The presence of beards is perceived to be associated with superior reproductive traits such as high conception, high prolificacy and high fertility rates (Gatew *et al.*, 2015). Large-scale studies are needed to establish the true effect of these qualitative traits on adaptation, performance and the overall productivity of Tswana goats.

Bodyweight and body sizes of Tswana goat have decreased compared with the previous report (Nsoso *et al.*, 2004). Nsoso and colleagues (2004) reported an average BW of 41.7 ± 0.5 kg and heart girth of 80.5 ± 0.3 in mature (>36 months) female Tswana goats. The difference could be due to a lack of availability of feed resources in quality and quantity. The current survey was undertaken during drought and there were substantial shortages of feed and water that could have major impacts on physical growth of animals. However, the indigenous mature Tswana goats had superior performance in terms of BW and body measurements compared with other indigenous goat breeds in Southern Africa (BW: 28.96 ± 0.37 kg, BL: 60.85 ± 0.29 cm) (Selolo *et al.*, 2015) and Tanzania (BW = 28.97 ± 0.52 kg, BL = 51.60 ± 0.40 cm) (Nguluma *et al.*, 2016). More studies on the effect of vegetation on performance should be performed for comparisons in and across agro-ecological regions of Botswana.

The availability of SNP arrays provides an opportunity to investigate current genetic structure and diversity of livestock for effective selection and conservation strategies (Groeneveld *et al.*, 2010). This study is the first attempt to genetically characterize the indigenous Tswana goat, using genome-wide SNP markers. The overall informative SNPs (92.5%) observed in the current study were higher than those reported by Lashmar *et al.* (2015) on Angora goats (82%) and Mdlala *et al.* (2016) on various South African indigenous goat breeds (87.1%), but comparable with Lashmar *et al.* (2016) on dairy goats (92.3%). The variation could be attributed to the threshold applied during QC. For instance, for Angora and South African indigenous goats a SNP call rate of 98% and 95% was used, respectively (Lashmar *et al.*, 2015 & Mdladla *et al.*, 2016) whilst in this study a SNP call rate of 97% was used. The results indicate, however, that the SNP chip could be used successfully for genomic studies on indigenous goats.

The results revealed high levels of genetic diversity (0.423) that is comparable with those by Kim *et al.* (2016) on the indigenous Barki goats (0.40) of Egypt and by Mdladla *et al.* (2016) on South African indigenous goats (0.41). Indigenous goats kept under communal systems are exposed to natural selection, where animals become genetically adapted for survival in their natural

environments, while maintaining high within and between population genetic variability (Kim *et al.*, 2016). Previous studies noted that the ancestor (bezoar) of the goat had broad genetic diversity, which has been conveyed to the modern goat, which has not undergone intensive selection, as has been experienced in species such as cattle (Gerbault *et al.*, 2012).

Individual inbreeding coefficient estimates in this study were lower than those reported (0.12 ± 0.16) by Maletsanake *et al.* (2013) using microsatellite markers on a Tswana goat population kept at an experimental farm under a controlled breeding programme. The difference could be because the samples in this study were drawn from a large population of communal areas, and care was taken to select unrelated individuals.

Principal component analysis revealed that most animals in the central region were clustered, with a few outliers. The reason for few outliers could be lack of selection and uncontrolled breeding being practised in communal management systems. The low value of cross validation ($K=1$) in this study is similar to the report of Kominakis *et al.* (2017) in Frizarta sheep. This indicates that there is no genetic differentiation or inferred clusters in the population. However, a more extensive sampling of Tswana goats covering various geographical regions of Botswana is necessary to assess the demographic history and relationship of individuals.

The average LD (0.067 ± 0.10) reported in this study is slightly lower than that in Mdladla *et al.* (2015), which reported values of 0.09 ± 0.12 on Zulu, Venda, and Xhosa and 0.11 ± 0.14 on South African Tswana goats. LD values, however, are influenced by factors such as history and structure of the population, sample size and strictness of SNP filtering (i.e. threshold of MAF and HWE) (Bohmanova *et al.*, 2011). Khatkar *et al.* (2008) pointed out that studies with relatively small sample sizes are subject to bias and loss of accuracy. This bias may vary with inter-marker distance. Therefore, it would be interesting to confirm the LD results in this investigation with a larger number of genotyped animals.

The pattern of LD decay with distance in this population is consistent with the report of Brito *et al.* (2015) on various breeds of goats. Linkage disequilibrium declined more slowly in the indigenous Tswana goat population than in the indigenous cattle population studied by Makina *et al.* (2014). The low level of long range LD indicates lack of selection on the current population or large effective population size in the recent past (Brito *et al.*, 2015). The persistence of LD over short distances, however, indicates that more markers will be needed to obtain the same power to detect association (Meadows *et al.*, 2008). Linkage disequilibrium decay determines the power of QTL detection in association mapping studies and helps to determine the number of markers required for successful association mapping and genomic selection (Mastrangelo *et al.*, 2014). Meuwissen *et al.* (2001) and Qanbari *et al.* (2010) proposed an r^2 value of ≥ 0.2 as being useful for association studies. Further advancement on dense SNP panel for goats would be essential for high power association mapping and genomic selection efficiency in future breeding programmes of Tswana goats.

The observed values of N_e in this study were consistent with those Mdladla *et al.* (2015) for South African ecotypes. Large effective population size is mostly correlated with natural selection and multiple breeding objectives (Meuwissen & Woolliams, 1994), which are the basic characteristics of smallholder communal goat production (Monau *et al.*, 2017). It is important to manage this effective population size to preserve genetic variability in the indigenous Tswana goat population for optimal utilization and sustainable development programmes.

Conclusions

Tswana goats possess environmental adaptive traits such as the presence of horns and beards, and mixed coat colour, and exhibit a relatively high level of genetic diversity and a large effective population size that can be exploited for future breed improvement. These are the first genomic data for the Tswana goat and could be used as a benchmark for further investigations. Further studies should be performed to genetically characterize a larger proportion of the indigenous Tswana goat breed for improved food security and increased productivity to benefit resource-poor farmers.

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Author's contributions

PIM and EMK conceived the study. PIM was the lead researcher performing data collection, data analyses, result interpretation and wrote the manuscript (as part of her PhD programme). CV, EMK & SJN provided research advisement, revised and the manuscript.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

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CHAPTER 5

Genetic diversity and population structure of indigenous goats based on Illumina Goat 50K SNP panel

Scientific paper prepared to be submitted for publication.

Genetic diversity and population structure of indigenous goats based on Illumina Goat50K SNP panel

Abstract

The aim of this study was to examine genetic variability and structure of indigenous Tswana and Swazi goats using Illumina Goat50K SNP Bead chip. Two South African commercial goat breeds were included in the analyses to investigate admixture with the indigenous populations. A total of 144 DNA samples which included Boer goats (n=24), Kalahari Red (n = 24), Swazi (n=48) and Tswana goats (n=48) were genotyped. Statistical analysis was performed using PLINK version 1.07. Genetic diversity, measured as expected heterozygosity was 0.390, 0.398, 0.413, and 0.387 for Boer, Kalahari Red, Tswana and Swazi, respectively. The average individual inbreeding coefficient across populations was 0.066 ± 0.07 . Principal component analysis and ADMIXTURE clustered the populations according to geographical origin and breed type. Linkage disequilibrium (LD) for shorter intervals (0-10 kb) ranged from 0.44 to 0.56. Effective population size decreased with generations and at the 13th generation was approximately 87 for Boer, 93 for Kalahari Red, 180 for Swazi and 266 for Tswana goats. Indigenous Tswana population have diverse pool of genetic diversity and large effective population sizes compared to other breeds that is important for breed development and conservation.

Keywords: Admixture, Breed diversity, Southern Africa, Tswana goats

Introduction

Goats are among the earliest livestock species to be domesticated approximately 11000 years ago near the Fertile Crescent of the Middle East (Zeder & Hesse, 2000). They are well adapted to different environmental conditions and are found in most parts of the world providing milk, meat and clothing material (Salles *et al.*, 2011; Agossou & Koluman, 2017). According to Food and Agricultural Organization (2016), there are approximately one billion goats worldwide with a substantial concentration in Asia (58.2%) and Africa (32.7%). In Africa, goat production mainly takes place in marginal areas under challenging environmental conditions with over 90% of goats categorised as indigenous breeds or types (Peacock, 2005). These indigenous breeds play an important role in ensuring food security and improving livelihoods in rural areas. They are mostly raised by rural communal farmers under extensive systems with minimum input and have evolved over time to adapt to the local environmental conditions such as poor quality forage, drought, diseases and heat (Gwaze *et al.*, 2009; Dzama, 2016). Their adaptive traits render them important as genetic reservoirs for future breeding programs (Huson *et al.*, 2014).

Southern Africa is a home to approximately 38 million goats (SADC, 2013) and most of the indigenous goats are assumed to have descended from various breeds of ethnic tribes in the north

around the 4th to 7th century AD (Smith, 1992). Given the different climatic and environmental conditions in the region, some of these populations have been isolated and exposed to different demographic forces such as selection pressures, genetic drift and gene flow, consequently, becoming genetically divergent (Ribeiro *et al.*, 2012). The genetic relationship between these indigenous populations is undocumented, probably due to poor definitions of breeds. For instance, Botswana and Swaziland have indigenous goats called Tswana and Swazi, respectively. The definitions of these breeds are often based on geographical areas in which the goats are found or ethnic groups keeping them, which do not reflect the genetic differentiation between goats (Hoffmann, 2010). Similar to other African countries, the Tswana and Swazi indigenous goats have never been selected for any production gains and are generally perceived to have slow growth rate, low mature weight, low carcass and milk yield and low productivity (Muema *et al.*, 2009; Mwai *et al.*, 2015). Consequently, they have been frequently exposed to indiscriminate crossbreeding and breed replacement with commercial breeds such as Boer and Kalahari Red goats (Garrine, 2007; Muema *et al.*, 2009). This has posed a threat to their existence and led to non-descript genotypes being predominant in low-input production systems (Tada *et al.*, 2013). Knowledge of the exact genetic relationship between Tswana and Swazi indigenous populations and their divergence from commercial goat breeds is still lacking, despite the risk of extinction. It is important to find ways of implementing optimal and sustainable genetic improvement programs without losing the adaptive traits of these indigenous genetic resources.

The use of molecular markers provides an opportunity to characterise breed specific information such as genetic diversity, genetic structure and genetic relationship within and between populations, which is relevant for breed monitoring and development, conservation and general improvement of goats (Hanotte & Jianlin, 2006). Molecular markers such as microsatellite markers have been extensively used with an effort to assess genetic erosion and assist in management strategies of indigenous goats (Groeneveld *et al.*, 2010; Lenstra *et al.*, 2012). Lately increased preference has been given to single nucleotide polymorphism (SNPs) markers that are robust, ubiquitous throughout the genome and highly polymorphic (Gama & Bressan, 2011). The Goat50K SNP array has recently been used to quantify genetic diversity and population structure of various indigenous goats in Africa including South Africa (Mdladla *et al.*, 2016), Sudan (Rahmatalla *et al.*, 2017), Ethiopia (Mekuriaw *et al.*, 2016) and Uganda (Onzima *et al.*, 2018). The Goat50K SNP array has also been used to differentiate Angora goats from Argentina, South Africa and France (Visser *et al.* 2016). However, few studies have been conducted on genetic relationship among indigenous goat breeds from different countries and across climatic areas of southern African region. As part of the current effort to characterise, conserve and design breeding strategies for indigenous goats, the aim of this study was to use Goat50K SNP chip to

investigate genetic variability and population structure of indigenous Swazi and Tswana goats and the influence of commercial goat breeds.

Materials and methods

Goat populations and DNA samples

The Botswana goat population was sampled in accordance with the Ethics Committee of Faculty of Natural and Agricultural Sciences at the University of Pretoria (ECO42-15). Farmers agreed to be part of the project by signing a consent form. Unrelated animals were selected based on information provided by farmers. Forty-eight (48) hair samples were collected from communal farmers in various villages of the Central region of Botswana. The samples were transported to the University of Pretoria laboratory and shipped to the Animal Genetic Laboratory in France, Labogena DNA platform (Domaine de Vilvert, CS 80009, 78353 Jouy en Josascedex) for DNA extraction and genotyping.

Ethical clearance for the use of external data for the South African commercial and Swazi populations was obtained from the South African Agriculture Research Council and University of Swaziland, respectively. The data included Boer goat (n=24), Kalahari Red (n=24) and Swazi goat (n=48) genotypes. The South African commercial breeds sampling and genotyping were described in details by Mdladla *et al.* (2016). In a nutshell, the animals were sampled from stud breeders and commercial farms. Blood samples were collected from Jugular vein using 6ml-EDTA vacutainer tubes. Blood samples were kept on ice and refrigerated at 4 degrees (°C). DNA extraction and genotyping was performed at Agricultural Research Council Biotechnology platform in South Africa., As for Swazi goats, hair samples from animal tails were collected from different agro-ecological regions of Swaziland and DNA extraction and genotyping was also performed at Agricultural Research Council Biotechnology platform in South Africa.

Genotyping and Marker-based quality control

All (144) animals were genotyped with the Illumina Goat50K Bead chip, which contains 53 347 SNPs distributed across the genome, with inter-SNP spacing of approximately 40kb (Tosser-Klopp *et al.*, 2014). Only mapped autosomal SNPs were considered for analyses, resulting in 49 943 SNPs. Sample and genotyping quality control (QC) was performed within breed and afterward on the merged dataset using PLINK version 1.07 (Purcell *et al.*, 2007). Individuals with missing genotype call rate of less than 93% were excluded from further analysis. The remaining individuals were then subjected to SNPs quality control. SNPs with a call rate of less than 95%, minor allele frequency of less than 0.05 and SNPs that deviated significantly from Hardy-Weinberg equilibrium (HWE; $P < 0.001$) were excluded from the analysis.

Statistical Analyses

The observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficient (F_{IS}) were determined using PLINK (Purcell *et al.*, 2007). The inbreeding coefficient (F_{IS}) was determined using the formula stated by Keller & Waller (2002): $F_{IS} = 1 - H_o/H_e$. Population structure and relatedness was estimated using principal component analysis (PCA) available with GCTA version 1.24 software (Yang *et al.*, 2011). Population structure was further explored to infer the most likely number of ancestral populations using ADMIXTURE version 1.23 (Alexander *et al.*, 2009). To determine the most optimal population structure using ADMIXTURE, a cross-validation method was presumed with assumed number of ancestral subpopulations from $K=2$ to $K=5$, as this could only provide clear biological reasons. Optimal partitioning of the population was achieved at the lowest cross-validation error.

To calculate linkage disequilibrium (LD), pairwise r^2 was assessed for each breed and chromosome using PLINK (Purcell *et al.*, 2007). The command ‘-r2 -ld-window-kb 2000 -ld-window-r2 0’ was used to estimate LD on SNP pairs up to a distance of 2000 kb. SNPs were grouped according to their pairwise distance into ten (10) categories: 0-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-200, 200-500, 500-1000 and 1000-2000kb and the average LD within each group was calculated. Means and standard deviation were computed using PROC MEANS on Statistical Analysis System (SAS Institute, 2009).

To estimate the effective population size (N_e) for each breed SNeP version 1.1 software (Barabato *et al.*, 2015) was used based on the formula described by Corbin *et al.* (2012). Minimum and maximum inter-SNP distances of 0 and 1000Mb were used, respectively. The data sets of each breed were grouped into 30 bins of 50kb distance. N_e estimates were then measured from r^2 values obtained for the mean of each bin.

Results

After quality control, only one animal from the Kalahari Red population was removed due to missing genotypes (--mind<0.07). A high number of SNPs were removed in different populations for various reasons; in the Boer population (3784) due to low MAF<0.05, in the Tswana population (1460) due to low call rate (<0.05) and in the Swazi population (142) due to significant deviance from HWE ($p<0.001$). The merged dataset included 143 animals and 46188 SNPs (Table 5.1).

Table 5.1 Number of animals and SNPs excluded during quality control in different goat populations

Population	n	Animals removed	Excluded SNPs			SNPs remaining
			MAF<5%	SNP CR <95%	HWE	
Boer	24	0	3784	788	51	45319 (90.7%)
Kalahari Red	24	1	3118	672	45	46107 (92.3%)
Tswana	48	0	1310	1460	53	47119 (94.3%)
Swazi	48	0	2453	675	142	46672 (93.5%)
Merged	144	1	1372	1239	1143	46188 (92.5%)

n=number of animals, CR- call rate, MAF- minor allele frequency, HWE- Hardy Weinberg equilibrium (P value <0.001)

The average MAF for the merged population was 0.319 ± 0.13 . The SNP markers showed a high level of polymorphicity in all populations, ranging from 92% to 97%. The expected heterozygosity (H_e) mean for all genotyped animals was 0.415 ± 0.02 . The inbreeding coefficients were generally low in all populations ranging from 0.011 ± 0.06 to 0.019 ± 0.05 (Table 5.2).

Table 5.2 Percentage of polymorphic markers, within-population diversity and inbreeding coefficient of different goat populations

Population	n	Polymorphic SNPs (%)	Average	$H_o \pm SD$	$H_e \pm SD$	$F_{IS} \pm SD$
			MAF $\pm SD$			
Boer	24	92	0.279 ± 0.14	0.384 ± 0.03	0.390 ± 0.01	0.014 ± 0.06
Kalahari Red	23	94	0.291 ± 0.14	0.393 ± 0.03	0.398 ± 0.01	0.012 ± 0.07
Tswana	48	97	0.318 ± 0.06	0.405 ± 0.07	0.413 ± 0.00	0.019 ± 0.05
Swazi	48	95	0.283 ± 0.15	0.383 ± 0.02	0.387 ± 0.02	0.011 ± 0.06
Merged	143	97	0.319 ± 0.13	0.388 ± 0.03	0.415 ± 0.02	0.066 ± 0.06

MAF- minor allelic frequency, H_o and H_e –observed and expected heterozygosity, F_{IS} - inbreeding coefficient

Principal components analysis accounted for 4% (PCA1) and 3.3% (PCA2) total variation and clustered populations according to geographical origins. The indigenous populations formed two independent clusters while South African commercial meat breeds clustered together with two outliers from Kalahari Red and one from Boer population (Figure 5.1).

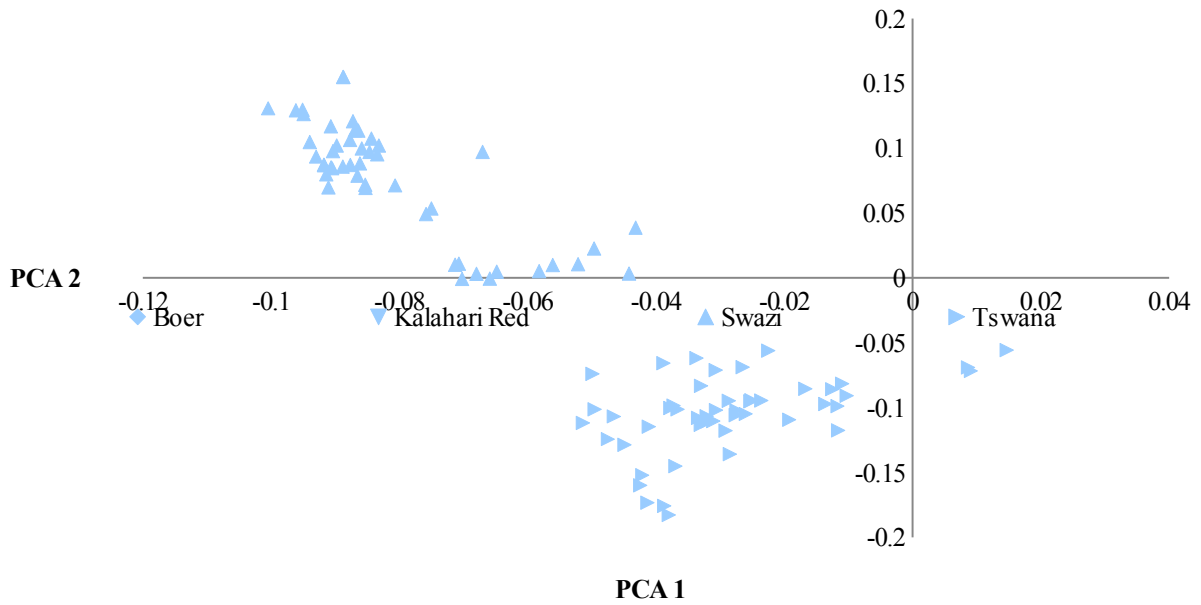


Figure 5.1 The genetic relationship between goat breeds using PCA analysis

The lowest cross-validation standard error (0.636) was assumed at K=3 (3 ancestral populations) signifying that this is the most probable number of ancestral breeds based on the data of this study. At K= 3, populations had distinct clustering according to their geographical areas represented by different colours (Green: South African commercial goats, Red: Swazi goats and Blue: Botswana population). There is admixture among the studied populations and indigenous Tswana and Swazi populations show common ancestry with commercial goats (Figure 5.2).

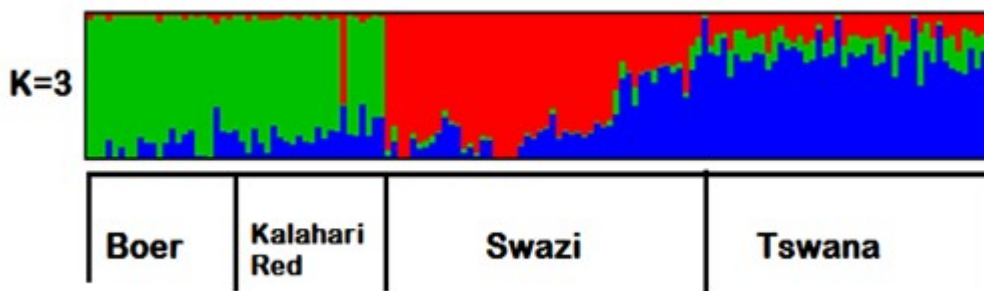


Figure 5.2 Population structures of Boer, Kalahari Red, Swazi and Tswana goats based on ADMIXTURE analysis

Information of the chromosomes length, number of SNPs and average linkage disequilibrium (r^2) per chromosome are summarised in Table 5.3. The dissemination of SNPs differed among chromosomes

from 825 to 3256. The overall r^2 across chromosomes ranged from 0.07 ± 0.11 to 0.19 ± 0.21 and Tswana population had the lowest r^2 values across chromosomes while commercial goats, especially Boer goat had higher r^2 values (Table 5.3).

Table 5.3 Summary of linkage disequilibrium (average r^2) per chromosome for Tswana, Swazi, Boer and Kalahari Red goats population.

Chr numbe r	Chr Length (MB)	Number of SNPs	r^2				
			Boer	Kalahari Red	Swazi	Tswana	Merged
1	138.87	3256	0.19±0.2	0.16±0.19	0.09±0.13	0.07±0.11	0.08±0.11
			1				
2	120.59	2830	0.19±0.2	0.16±0.19	0.09±0.13	0.07±0.11	0.07±0.11
			1				
3	100.93	2381	0.20±0.2	0.16±0.19	0.09±0.13	0.07±0.11	0.07±0.11
			1				
4	102.59	2416	0.17±0.2	0.14±0.19	0.10±0.13	0.07±0.11	0.07±0.11
			1				
5	95.09	2244	0.18±0.2	0.15±0.19	0.09±0.13	0.07±0.11	0.07±0.11
			1				
6	103.28	2438	0.24±0.2	0.21±0.19	0.09±0.13	0.08±0.11	0.09±0.11
			1				
7	92.47	2192	0.22±0.2	0.18±0.19	0.09±0.13	0.07±0.11	0.08±0.11
			1				
8	99.73	2352	0.22±0.2	0.18±0.19	0.09±0.13	0.07±0.11	0.08±0.11
			1				
9	80.05	1895	0.18±0.2	0.16±0.19	0.09±0.13	0.07±0.11	0.07±0.11
			1				
10	91.02	2099	0.17±0.2	0.14±0.19	0.08±0.13	0.06±0.11	0.06±0.11
			1				
11	92.61	2139	0.18±0.2	0.17±0.19	0.09±0.13	0.07±0.11	0.07±0.11
			1				
12	75.91	1750	0.22±0.2	0.19±0.19	0.09±0.13	0.07±0.11	0.08±0.11
			1				
13	71.12	1650	0.20±0.2	0.19±0.19	0.09±0.13	0.06±0.1	0.07±0.11
			1				
14	82.40	1912	0.20±0.2	0.18±0.19	0.08±0.13	0.07±0.1	0.08±0.11
			1			0	
15	70.66	1640	0.19±0.2	0.15±0.19	0.09±0.13	0.06±0.11	0.07±0.11
			1			0	
16	68.64	1593	0.20±0.2	0.14±0.19	0.08±0.13	0.07±0.1	0.07±0.11
			1			0	

17	63.64	1470	0.19±0.2	0.15±0.19	0.08±0.13	0.07±0.1	0.07±0.11
			1			0	
18	55.87	1292	0.20±0.2	0.17±0.19	0.09±0.13	0.08±0.11	0.08±0.11
			1				
19	53.31	1228	0.19±0.2	0.16±0.19	0.09±0.13	0.06±0.11	0.07±0.11
			1				
20	64.70	1496	0.18±0.2	0.17±0.19	0.11±0.13	0.08±0.1	0.09±0.11
			1			0	
21	61.80	1431	0.16±0.2	0.14±0.19	0.08±0.13	0.06±0.11	0.06±0.11
			1				
22	50.48	1170	0.20±0.2	0.14±0.19	0.08±0.13	0.07±0.11	0.07±0.11
			1				
23	45.42	1048	0.16±0.1	0.15±0.19	0.07±0.13	0.05±0.11	0.05±0.11
			7				
24	56.81	1324	0.21±0.2	0.17±0.19	0.09±0.13	0.07±0.11	0.07±0.11
			1				
25	36.95	856	0.20±0.2	0.17±0.19	0.08±0.13	0.07±0.1	0.08±0.11
			1			0	
26	45.09	1045	0.18±0.2	0.15±0.19	0.08±0.13	0.06±0.11	0.06±0.11
			1				
27	40.07	929	0.18±0.2	0.14±0.19	0.09±0.13	0.07±0.1	0.07±0.11
			1			0	
28	39.41	915	0.18±0.2	0.15±0.19	0.07±0.13	0.06±0.11	0.06±0.11
			1				
29	42.10	978	0.18±0.2	0.15±0.19	0.07±0.13	0.06±0.11	0.06±0.11
			1				
Overall	2141.54	49943	0.19±0.2	0.16±0.19	0.09±0.13	0.07±0.11	0.07±0.11
	7		1				

Chr = Chromosome

Levels of pairwise linkage disequilibrium (LD) decreased with increasing distance between SNP pairs in all breeds. For shorter interval range of 0-10 kb LD levels ranged from 0.56-0.44 and commercial breeds had higher values (Figure 5.3).

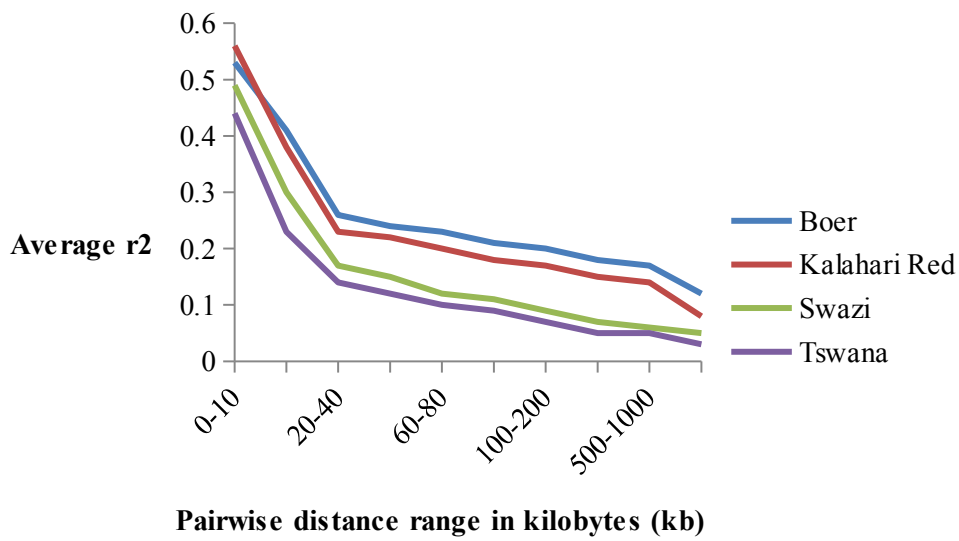


Figure 5.3 Decay of LD by distance for Boer, Kalahari Red, Swazi and Tswana goat populations

The estimates of effective population size (N_e) were evaluated per breed-group approximately from 983 to 13 generation ago. All populations followed a similar trend whereby N_e decreased over time. At the 13th generation ago, the effective population size per breed-group was approximately 87 for Boer, 93 for Kalahari Red, 180 for Swazi and 266 for Tswana goats, respectively (Figure 5.4).

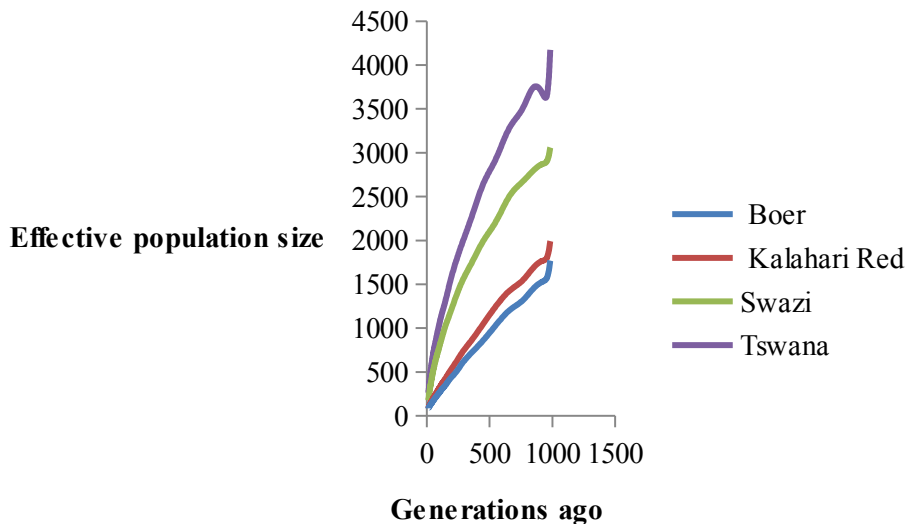


Figure 5.4 The effective population size of Swazi, Tswana and commercial breeds from 983 to 13 generations ago.

Discussion

Indigenous goats contribute considerably to the sustenance, financial and social livelihoods of rural communities. These goats possess distinctive characteristics due to natural selection (Dzama, 2016). Despite this, most of these animals are neglected in terms of scientific research, breed development and often exposed to aimless crossbreeding with exotic or commercial breeds (Abegaz, 2014). The loss of these breeds or reduction on their genetic diversity will definitely limit the prospects of food security and future breeding programs especially in developing countries (FAO, 2012). In this study, the Goat50K SNP chip was used to examine genetic diversity and population structure of two southern African indigenous goats (Tswana and Swazi) and the possible influence of two commercial breeds (Boer and Kalahari Red).

Quality control procedures removed a higher number of SNPs in Boer population due to low minor allele frequency ($Maf < 0.05$). This could indicate that Boer population sampled in this study is subjected to high intensive selection as compared to other populations. More markers were also removed in Swazi population due to deviation from Hardy-Weinberg equilibrium, which could be due to genetic drift or population subdivisions (Wahlund effect) (Kantanen *et al.*, 2000). The proportion of polymorphic markers indicates that most SNPs used in the development of Goat50K SNP array are segregating within the investigated breeds and the chip can be used across different goat breeds (Mdladla *et al.*, 2016; Onzima *et al.*, 2018). The advancement of Goat50K SNP array covered a wide variety of breeds from different types and origins (Tosser-Klopp *et al.*, 2014). A comparable degree of polymorphism has been reported in various indigenous goats including 84 to 98% in South African goats (Mdladla *et al.*, 2016), 96.8 to 99.7% in Italian goats (Nicoloso *et al.*, 2015), 96% in Sudanese goats and 89 to 93% in Ugandan goats (Onzima *et al.*, 2018).

The general high levels of genetic diversity measured as expected heterozygosity (H_e) in this study are comparable to South African ($H_e = 0.37-0.41$) (Mdladla *et al.*, 2016), Sudanese ($H_e = 0.39-0.40$) (Rahmatalla *et al.*, 2017) and Ugandan indigenous populations ($H_e = 0.38-0.41$) (Onzima *et al.*, 2018). Generally, goats have high genetic diversity compared to other species such as cattle, indicating less bottlenecks, selection and breed diversification during and post domestication (Bovine HapMap Consortium, 2009). The observed genetic diversity in the studied populations provides opportunities for conservation and breeding strategies aimed at improving traits through heterosis and within-breed selection (Kantanen *et al.*, 2000). In commercial goat breeds the opportunities to increase the output per animal using selection has been confirmed (Facó *et al.*, 2011). Indigenous Swazi and Tswana goats could also be improved through within-breed selection, which allows sustainable utilization ultimately securing conservation of these breeds (Mueller *et al.*, 2015). For short-term results, well-designed crossbreeding programmes have shown incredible findings, particularly for meat production with different species (Rege *et al.*, 2011; Mokolobate *et al.*, 2014). The results further reveals that the

studied populations have been out-bred, which could be linked to the observed admixture and high genetic diversity. A low inbreeding coefficient has also been reported in Angora goats (0.08) (Visser *et al.*, 2016), dairy and fibre goats (0.08) (Lashmar *et al.*, 2016) and South African indigenous goats (0.07) (Mdladla *et al.*, 2016). It is therefore, important to maintain the low levels of inbreeding and high genetic diversity on these populations to allow future genetic improvement and response for any endeavours of climate change.

The goat populations were well differentiated according to geographical origin and breed type, which is consistent with several authors (Mdladla *et al.*, 2016; Visser *et al.*, 2016; Onzima *et al.*, 2018). Although the animals separated geographically, there are genetic links among the studied populations. This could be due to co-ancestry, crossbreeding or gene flow. The indigenous populations from two countries descended from goats in the north of Africa and took similar routes of migration (Smith, 1992). In addition, movement of animals between these countries is not prohibited therefore gene flow could be possible due to livestock migration and trading. Indigenous goats are also kept under communal management system where there are no breeding objectives and uncontrolled mating is a common phenomenon (Gwaze *et al.*, 2009; Monau *et al.*, 2017). There is a possibility that indigenous populations are diluted, which causes concern as adaptive traits may diminish. It is therefore, of utmost importance to conserve these breeds for the upkeep of smallholder farmers who benefit from them.

All populations studied showed low levels and rapid decay of linkage disequilibrium (LD) which can be associated with small sample size. Khatkar *et al.* (2008) pointed out that reports with relatively small sample size are imperilling to bias and imprecision, and this bias may vary with inter-marker distance. Bohmanova *et al.* (2010) suggested that for Holstein cattle breeds a minimum 55 individuals should be used to avoid underestimation or overestimation of r^2 . Fewer animals were genotyped in the study therefore these results must be interpreted with caution. There was quite small variation in LD levels per chromosome in commercial goats whilst very little to no variation was observed among indigenous goats. Lack of variation in LD measures between chromosomes has previously been reported on Zimbabwean indigenous goats (Zvinorova, 2017), which indicates lack of intensive selection. The small variation of LD levels per chromosome on commercial breeds indicates that intense selection pressure was applied on polygenic traits (Brito *et al.*, 2017). The difference in LD measures between chromosomes can be explained by variation in autosomal recombination rates, heterozygosity, genetic drift and effects of selection (Arias *et al.* 2009; Qanbari *et al.*, 2010). Furthermore, the difference of LD between breed-groups could also be linked to recent and past history of selection and effective population sizes (small population size is correlated to higher LD) (Brito *et al.*, 2015). Previous studies postulated that LD in indigenous breeds persists for relatively shorter range, which can be attributed to highly heterogenous populations and low selection intensity

over generations (Makina *et al.*, 2014; Mdladla *et al.*, 2016; Zvinorova, 2017). The low levels of LD observed in this study have practical implications on the implementation of genome-wide association studies such as genomic selection. It underlines the need to use a denser panel for the indigenous goats and a large sample size or training population for commercial breeds.

The effective population sizes (N_e) of the studied goat populations have decreased overtime due to natural and artificial selection. Similar trends have been reported by Mdladla *et al.* (2016) and Brito *et al.* (2017). Indigenous Swazi and Tswana populations have been naturally selected, based on survival to unpredictable harsh and changing environmental conditions (Kim *et al.*, 2016). A threshold of $N_e=100$ has been suggested to guarantee long-term viability of livestock populations (Meuwissen, 2009). The effective population size for indigenous populations was above the suggested threshold, indicating high genetic variability of these populations that is suitable for the achievement of high selection and genetic response in the long-term (Goddard, 2009). This provides prospects for effective conservation programmes for these breeds and future genomic programmes that will promote global food security. The N_e depression observed around 900 years ago could be due to bottlenecks associated with breed formation and/or selection. The lower effective population size observed in the commercial breeds is consistent with the findings of Mdladla *et al.* (2016) and Visser *et al.* (2016). This could be the consequence of continuous artificial selection, which will ultimately lead to narrow genetic pool and inbreeding. Interventions such as exchange of breeding bucks between stud breeders or artificial insemination should be considered, as the repercussions may lead to decreased fitness and low reproduction or survival rate (Tada *et al.*, 2013).

Conclusions

The studied populations exhibited genetic diversity, which is important for adaptation to ever-changing environments, conservation and breeding programs. The goat populations clustered according to geographical origin and breed type. There is co-ancestry among the populations and gene flow could have facilitated the observed admixture. The large effective population size in the indigenous population should be maintained to preserve within-breed genetic diversity. Only two indigenous populations were evaluated in this study. It is therefore recommended that more goat populations should be sampled and studied in the southern African region. Carrying out comparative evaluations with indigenous populations from other countries may provide more insight on the ancestry and structure of these animals. This may assist in decisions concerning breed conservation and general goat productivity.

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Statement of Interest

The authors declare that they have no conflict of interest.

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CHAPTER 6

Critical Review, Recommendations and Conclusions



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Critical Review

Livestock production is an important socio-economic activity in Botswana. Most of the livestock are kept in the communal land under traditional management systems (Darkish & Maida, 2002). Cattle dominate the livestock industry with 58%, followed by goats with 35% and sheep with 7% (Statistics Botswana, 2013). Goats have a key role in ensuring food security to smallholder farmers especially in rural areas. Indigenous goats in particular are more important due to their adaptive traits to local environments and low maintenance (Nsoso *et al.*, 2004; Visser, 2017). However, poor breed documentation, uncontrolled crossbreeding, poor management and disorganised breeding structures remain a threat to the indigenous goats (Rischkowsky *et al.*, 2007). Characterisation of local genetic resources is a rational national strategy for improving knowledge of the country's animal genetic resources (AnGR). It is also an important prerequisite for developing breeding strategies and conservation programmes for AnGRs (FAO, 2011). In this thesis, the focus was on the indigenous Tswana goats in context of the relevant production systems, phenotypic and genetic characterisation. The genetic relationship of Tswana goat with other local and commercial breeds in the region was also examined.

The production of the indigenous Tswana goats is subsistence oriented fulfilling multiple functions that contribute to food security of the resource poor farmers keeping this breed. Similar to other studies in Africa (Kosgey *et al.* 2006; Abegaz, 2014), there are several challenges hindering goat productivity in Botswana such as lack of water and feed resources, prevalence of diseases, predation and lack of record keeping (Kosgey *et al.*, 2008). These challenges impede the prospect to increase and sustain food production in rural areas. In the past, some programmes have been employed to improve goat productivity, including nucleus breeding schemes (open and closed) and crossbreeding. These approaches failed to provide sustainable solutions due to lack of sustainable support from governments and donors and inadequate engagement of the intended users, namely the smallholder farmers (Kosgey *et al.*, 2006).

Community based breeding program has emerged as a promising option to improve goat productivity in communal production systems (Haile *et al.*, 2011). The programme considers the production system and takes into account the indigenous information on breeding practices and breeding objectives. It also involves the local community at every level from planning to operation of a breeding program (Gizaw *et al.*, 2013). Such a program has been practised in smallholder farmers of Ethiopia since 2007 where it yielded positive results such as increased productivity, 3% reduction in inbreeding, increased chevron/mutton consumption, increased capital and sustainable utilization of indigenous breeds (Gutu *et al.*, 2015). A program of this nature could potentially be implemented in Botswana as well, given the similarity of smallholder production systems in sub-Saharan Africa.

The flock structure of the surveyed regions shows reduction in population size of the indigenous Tswana goat. This is due to challenges previously stated and the condition is aggravated by absence of records and unplanned mating systems, which is a common habit in the smallholder communal system. Farmers preferred crossbred or exotic breeds to indigenous Tswana goats due to better growth rate and high market value even though they do not perform well under communal management systems. Any decline in the diversity of genetic resources tapers the possibility to react to environmental and climate changes or demand patterns (Hoffman, 2010), which will ultimately undermine food and livelihood security of the poor to survive in marginal areas. This underlines the need to take immediate steps to conserve this breed. Most of the genetic diversity studies of indigenous livestock genetic resources in Africa have recommended conservation through sustainable utilisation (Alemu, 2004; Garrine, 2007; Mogesse, 2007). However, implementations have been minimal due to lack of funds, political instability, lack of workable agricultural policy and lack of cooperation from farmers (Nyamushamba *et al.*, 2017). Successful preservation of indigenous livestock biodiversity relies, largely, on governmental policy. Policy makers need to be educated about the importance of animal genetic resources (AnGR). This will result in governments allocating more resources to conservation of AnGRs. The conservation expenditure can be tackled by raising the market value of indigenous livestock and their products so that they ultimately turn out to be self-sustaining (Oldenbroek, 2007). Furthermore, cross breeding under communal setup should be banned and practised only under well-structured or controlled management system with proper infrastructure (e.g. fenced areas). This can be achieved through proper governmental laws and regulations hence assist Tswana goat preservation.

The government of Botswana has made initiatives to conserve indigenous livestock species and Tswana goats are maintained as purebred flocks on government ranches (Mpofu, 1996). This offers prospects for utilisation, breed evolution and development and maintenance of production (FAO, 2000). Programs, structures and modalities to implement conservation with sustainable utilization are however, still lacking. In addition, given that most local breeds tend to have small effective populations the risks of inbreeding and genetic drift are always high, and small populations tend to be at risk to unexpected catastrophes (Rischkowsky, 2007). Sustainable agricultural systems emphasise on conserving indigenous breeds to their environment particularly with the communities who keep them (Mueller *et al.*, 2015). Once utilization has been established, farmers should be encouraged to preserve indigenous Tswana goats through formation of breeder associations. Once again, the government can formulate policies that incentivise farmers to keep AnGRs. Farmers can be taught on performance record keeping for various traits and extension workers can monitor the progress.

Tswana goats have been properly classified based on morphological data countrywide (Nsoso *et al.*, 2004). Dependence on these data as the basis for classification for utilisation and/or conservation is

however, generally subjective (Okomo-Adhiambo, 2002). Genetic characterisation of indigenous Tswana goats based on DNA studies is essential, as it is more consistent and it is based on exact genotypic information (Ajmone-Marsan *et al.*, 2014). It is unfortunate that only one part of the central region of Botswana was genetically characterised in this study. The lack of data from other regions could give an incomplete genetic variation and distorted picture of the population structure of Tswana goat countrywide. Nevertheless, this is the first study on genomic work on Botswana livestock and results have revealed high genetic diversity on indigenous Tswana goats, which can be used as a guide for conservation, genetic improvement through within-breed selection and genome wide association studies.

Another important component for conservation decisions is the level of genetic variation among breeds in the Southern African region (Nyamushamba *et al.*, 2017). The goats in the region have different names and geographical habitats but show similar phenotypes. The unselected Tswana and Swazi populations from Botswana and Swaziland are genetically linked and share the same genetic background and/or could have undergone the same selection pressures, even though separated geographically. These goats followed the same migratory route from Bantu tribes in the north before and after the arrival of European settlers (Smith, 1992). They are kept under traditional management system where natural selection is a common phenomenon. The study confirmed that these breeds are ecotypes. An establishment of conservation programmes in two countries is vital. These countries have organizations such as government research centres and universities, which can spearhead the conservation through breed utilization of indigenous goats.

Recommendations

This small but pioneering study provided new insights on genetic and phenotypic variation and their implications on conservation of the indigenous Tswana goat breed. It will undoubtedly form the basis of future larger studies covering all geographical regions of Botswana. This will provide a national representation of genetic diversity of indigenous Tswana goats. To obtain accurate information on genetic characterisation of indigenous goats, an average sample size of 40 animals per region is recommended (Okomo-Adhiambo, 2002). This will assist in decision making for conservation such as the method to be used and economic practicability. The most practical recommended method for conserving indigenous goats in Botswana is by *in situ* method through community-based conservation program (Gibson *et al.*, 2006).

Furthermore, conservation of livestock resources should ideally be undertaken at global or regional level because of the existence of trans-boundary breeds (Gizaw, 2011). This can inform sustainable regional conservation and utilization efforts.

Given the genetic diversity reported in this study the Tswana goat has the potential to be improved through within-breed selection programme. Mueller *et al.* (2015) stated that within-breed selection of the adapted indigenous genotypes is a feasible and assuring plan for increased productivity, efficient, sustainable on-farm conservation and use, which guarantees support to the economy of communities depending on them. Abegaz (2014) provides more details on how this can be achieved in communal productions system in developing countries. Setting up of recording schemes will be key and this can be done by farmer training and sustained support from the government.

Current goat improvement schemes in Botswana by public, private and non-governmental organisation (NGOs) actors tend to be sporadic and poorly planned. More concerning is their preference of exotic goats over indigenous goats. These goats usually do not perform well under low-input production systems in communal areas. It is recommended that indigenous goats should be selected at the research institutions and the best males can then be disseminated to smallholder farmers/cooperatives to improve productivity and at the same time promote conservation of indigenous genetic resource. It would be interesting to look at the performance of different ecotypes in different environments. Larger studies on geographical and production parameters such as milk, meat, growth and reproduction of Tswana goat are needed for holistic characterisation and strategic planning for more efficient utilisation of Tswana goats.

Future development of high-density goat SNP panels will permit the examination of indigenous Tswana goat genome at a very high resolution. Using a denser SNP marker panel increases linkage disequilibrium (LD) between the markers that will assist in marker-trait associations in genome-wide association studies (GWAS) (Mdladla, 2016). Studies regarding association and linkage disequilibrium tests and gene ontology are paramount in Tswana goats, as they have been employed to identify some of the genetic variants related to adaptability, disease resistance, heat resistance, response to medication and other important traits in other livestock species (Mohlatlole *et al.*, 2015). A genome approved could be used to aid in sustainable breeding improvement and development programs for indigenous goats. There are a number of opportunities for genomic applications in research, but to date it remains at an infancy stage in Botswana.

Conclusions

This study confirms that the Tswana goat is an important genetic resource kept under resource-poor farming systems with potential for increased productivity to enhance food security and the welfare of the nation. These genetic resources have become even more important under changing climate. The genetic and phenotypic diversity reported in this study can result in genetic gain if selection is applied to these populations. However, given that the majority of farmers, who own indigenous goats are resource poor, sustained government support is required for the success of breeding improvement

programs. The Tswana and Swazi goats are ecotypes therefore, regional genetic improvement programs should be set up to benefit farmers who keep this adapted genetic resource.

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APPENDICES

Research subject information and consent form

Title: Genomic Characterisation of Tswana goats

Name of Researcher: Phetogo Monau (PhD student)
Student number 15096883
University of Pretoria
South Africa

Purpose of questionnaire

Goat production in Botswana has great potential for food security and income generation of resource-poor farmers. In order to effectively design sustainable genetic improvement programs, the importance of goats to farmers, their genetic and production characteristics need to be evaluated and clearly understood. The success of any genetic improvement and conservation programme depends upon the action of livestock keepers who own, utilize and adopt breeds and adapt them to their needs. Therefore, the aim of this survey is to understand goat production systems, to understand the importance of goats to the farmers and identify breeding practices and constraints encountered in goat production of Botswana.

Description of the study

The questionnaire will be administered in different agro-ecological regions of Botswana; Southern region, Central region, North-west region, Okavango delta and Ghanzi/Kgalagadi region. Three districts and three villages within the region will be randomly selected. Information concerning the number of goats per region will be taken from regional agricultural extension officers. At least 50% of households keeping goats will be randomly approached and interviewed. The questionnaire will address the following: demography, size of land and ownership, livestock kept on the farm, production system, management practices, purpose of keeping goats, breeding practices, production constraints and common diseases at farm level. The questionnaire will be administered to the household head or any other mature person (if the household head is not available) using Setswana or English language.

Expected outcomes

The research will be able to provide information on;

- Distribution of goat breeds, breed status and their physical characteristics in Botswana.
- Goat breeds preferred by farmers and their local uses in Botswana

- Recommendations on the improvement of goat production in Botswana.

CONSENT FOR PARTICIPATION IN A RESEARCH INTERVIEW

Research Title: Phenotypic and genetic characterisation of Tswana goats in Botswana

Name of Researcher: Phetogo Monau (PhD student)
Student number 15096883
University of Pretoria
South Africa

- ❖ I volunteer to participate in a research project conducted by Phetogo Monau from University of Pretoria. I understand that the project is designed to gather information about goat production in Botswana. I will be one of approximately 250 people being interviewed for this research.
- ❖ My participation in this project is voluntary and I understand that I will not be paid for my participation. I am free to refuse to participate and I am free to withdraw from the interview at any time. My refusal to participate or withdrawal of consent will not affect my treatment or my relationship with the University of Pretoria.
- ❖ I understand that the data collected from my participation will be used for purpose (e.g. thesis, and journal publication), and I consent for it to be used in that manner.
- ❖ I have read and understand the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study.

Name (please print) _____

Signed _____

Date ___ / ___ / ___

Goat production in Botswana-Questionnaire

Goat production in Botswana has great potential for food security and income generation of resource-poor farmers. In order to effectively design sustainable genetic improvement programs, the importance of goats to farmers and their genetic and production characteristics need to be evaluated and clearly understood. The success of any genetic improvement and conservation programme depends upon the action of livestock keepers who own, utilize and adopt breeds and adapt them to their needs. Therefore, the aim of this survey is to understand goat production systems, to understand the importance of goats to the farmers and identify breeding practices and constraints encountered in goat production of Botswana.

DEMOGRAPHIC INFORMATION

Name _____

Surname _____

Address _____

Location _____

Village _____

Districts _____

Region _____

Farm name _____

HOUSEHOLD STRUCTURE

Farm Type a) Large scale-commercial b) small-scale commercial c) commercial

Land ownership a) own b) lease c) other (Specify) _____

Head of household a) Male b) Female

Position in the household a) head of household b) spouse c) son d) daughter e) brother
f) sister g) other (specify) _____

Age a) ≤30 b) 31 -45 c) 46 -60 d) ≥ 61

Marital Status a) Married b) Single

Level of education a) Primary b) Secondary c) Tertiary d) None

Number of people living in the household

Male Female Children < 15 years

Is livestock major activity a) Yes b) No

Source of income (Please tick where applicable and rank according to importance)

Activity	Tick	Rank 1,2,3
Salary/wages		
Crops		
Livestock products		
Home industries		
Other (Specify)		

Livestock Kept (Write total number for each species kept and rank according to importance)

Livestock	Number	Rank (1,2,3)
Cattle		
Goats		
Sheep		
Chicken*		
Pigs		
Donkeys		
Other		

*adults birds only

Livestock production category

meat	milk	Dual purpose
Cattle	Cattle	Cattle
Sheep	Sheep	Sheep
Goat	Goat	Goat

Trend in livestock population and land holding status over lifetime; *please circle where appropriate*

Cattle	sheep	goat	Land holding status
a) Increase	a) Increase	a) Increase	a) Increase
b) Decrease	b) Decrease	b) Decrease	b) Decrease

c) No change	c) No change	c) No change	c) No change
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Reasons for decline in land holding a) drought b) increase in population c) Diseases d) lack of funds e) no idea f) other.....

Reasons for increase in land holding a) water availability b) pasture availability c) availability of funds d) less diseases e) other _____

Flock Management

Do goats run with other livestock a) Yes b) No

GOAT PRODUCTION

Type of production system a) Intensive b) semi-intensive c) Extensive/ pastoral d) free-range/backyard e) other _____

Purpose of keeping goats (Tick and Rank, 1 for primary purpose, 2 for secondary,3 for third purpose)

Purpose	Tick	Rank
Meat		
Milk		
Skin		
Cash from Sales		
Ceremonies		
Breeding		
Insurance/emergency		
Cultural rites		
Investment		
Manure		

Members of household responsible for goat activities (*Tick where necessary*)

Activity	Male adult	Female adult	Boys (<15)	Girls (<15)	Hired Labour
Feeding					
Milking					
Breeding decisions					
Slaughtering					
Selling					
Purchasing					
Animal Health					
other					

Feeding

Source of feeding	Dry season (tick)	Wet season (tick)	Feed supplement	Dry season (tick)	Wet season (tick)
Natural Pasture			Roughage		
Hay			Concentrate		
Crop residue			Minerals / vitamin		
Yard			Licks		
Tethered			None		
Other _____			Other _____		

Watering

How animals are watered (tick)	Dry season	Wet season
Animals go to water		
Water is fetched		
Both		
Source of water (tick)	Dry season	Wet season
Borehole		
River		
Pond		
Rain water		
Tap water		
Other		
Distance to watering point	Dry season	Wet season
In the household		
<1km		
1-5 km		
6-10 km		
>10 km		

Frequency of watering (Tick)	Dry season	Wet season
Adlibitum		
Once a day		
Twice a day		
Every other day		
Once in 3 days		
Other		
Water quality	Dry season	Wet season
Good/clear		
salty		
smelly		
Other		

Housing

Method of housing	Dry season	Wet season
		Tick
Materials used for housing		
Wire		
Mud		
Bricks		
Untreated wood/bush		
Treated wood		
Iron sheets		
Other _____		
Form of housing	tick when present	
Roofed		
Solid wall		
Concrete floor		
Wooden floor		
Earth floor		
Other		

Herd Health

Access to veterinary services a) Government vet b) Private vet c) veterinary drug supplier
 d) Extension service e) None f) other _____

Common Diseases or symptoms seen (Rank according to most common)

Treatment of Diseases (If known)

Prevalence of diseases a) dry season b) wet season c) all year round

Vaccinations and preventative medicines given a) Yes b) No

If yes fill the table below and tick appropriately

Name Diseases/symptoms	Done routinely	Done when need

External parasites control a) Yes b) No (*If yes fill the table below*)

Method	tick	Done routinely		Done when need		If done routinely specify how often	
		Dry season	Wet season	Dry Season	Wet season	Dry season	Wet season
Spray							
Pour-on							
Dip							
Hand-dressing							
Injectables							
Traditional							
Other							

Specify traditional

Endoparasites control a) Yes b) No (*If yes complete the table below*)

Method	tick	Done routinely		Done when need		If done routinely specify how often	
		Dry season	Wet season	Dry Season	Wet season	Dry season	Wet season
Drench							
Traditional							
Injectable							
Other							

Castration

Do you castrate? a) Yes b) No

Age of castration a) 0-3months b) 3-6 months c) 6-12months d) 12-24months

Method of castration a) knife b) rubber ring c) badizzo

Reasons for castration a) improve meat quality b) improve temperament c) control breeding d) other _____

Marketing and culling

Do you cull a) Yes b) No (If yes complete the table below)?

Reasons	Male	Rank	Female	Rank
Temperament				
Diseases				
Old age				
deformities				
Poor Performance				
Poor fertility				
Body size				
Body conformation				
Other				

Do you sell? a) Yes b) No

How often do you sell?

Where do you sell a) Traders b) Consumers c) Farmers e) auctions e) abattoirs
f) other....

When do you sell? a) Holidays b) Ceremonies c) during drought d) shortage of food commodities e) other _____

Sex of animals sold a) entire males b) castrates c) females d) mixed

Age of animals sold a) ≤ 6 months b) 7-12 months c) 12-18 months d) 18-24 months e) 24-36 months f) >36 months

Reasons for selling _____

Breeding

Mating a) uncontrolled b) group mating c) hand mating e) other _____

Source of breeding buck a) Borrowed b) hired c) bought d) Own herd e) communal area buck f) other _____

Type of buck (breed name, if known) _____

Reasons for choice of buck	Tick	Rank 1,2,3
Body conformation/shape		
Body size		
Colour		
Performance		
Availability (no choice)		
Other		

How long do you use the buck for breeding? a) 1-2 years b) 2-4 years c) ≥ 5

Breed of goat kept a) indigenous b) pure exotic c) indigenous x exotic crosses d) exotic crosses

Flock structure of goats kept; circle where applicable number kept

Kids (≤ 4 months)	Weaners (5-12 months)	Adults (>12 months)
a) ≤ 10	a) ≤ 10	a) ≤ 10
b) 10 – 20	b) 10 – 20	b) 10 – 20
c) 21 – 40	c) 21 – 40	c) 21 – 40
d) 41 -50	d) 41 -50	d) 41 -50
e) >50	e) >50	e) >50

Origin of goats kept a) bought b) inherited c) Gifts d) acquired through govt scheme e) exchanged f) other _____

Quality of your animals (farmer's perception) rate them according to (Poor, average & good)

Trait	Male			Female		
	Poor	Aver	Good	Poor	Aver	Good
Growth rate						
Body size						
Meat						
Milk						
Prolificacy						
Disease tolerant						
Drought tolerant						
Heat tolerant						
Temperament						
Body shape						
colour						
fertility						
Other						

Record keeping a) Yes b) No

What kind of records do you keep? _____

Reproductive performance

Age of male puberty a) 6-12 months b) 12- 18 months c) 18- 24 months

Age of female puberty a) 6-12 months b) 12- 18 months c) 18- 24 months

- Age at first service a) 6-12 months b) 12- 18 months c) 18- 24 months
- Age at first kidding a) 6-12 months b) 12- 18 months c) 18- 24 months
- Lambing interval a) 6- 8 months b) 8- 12 months c) 12-18 months d) [>]18months
- Average reproductive life span a) 3-5 years b) 6-8 years c) 9-11 years d) \geq 12
- Twinning rate a) \geq 50% b) 60%- 70% c) 70%-90%
- Weaning Practices a) Natural b) artificial c) other _____
- Weaning age a) 2-4 months b) 4-6 months c) 6-8 months d) 8-12 months

Major constraints

List the constraints encountered according to their occurrence:

ANIMAL AND SAMPLING INFORMATION

- Breed a) Indigenous b) exotic c) crossbred
- Age by dentition a) \leq 1year b) 1-2years c) 2-3 years d) 3-4years e) \geq 5 years
- Sex a) entire male b) female c) castrate

Phenotypic characteristics

Trait	Measurement
BW	
BL	
HW	
HG	
TL	
Colour	
Horned	Yes/ No
Beard	Yes/ No

BW= body weight; BL= bodylength; HW= height at withers
HG= heart girth; TL= tail length

Type of biological sample a) Blood b) hair

Area collected _____

Date of collection _____

Name of collector _____

