Genetic diversity and population structure of South African dairy goats

By

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Declaration

I declare that the thesis/dissertation, which I hereby submit for the degree MSc (Agric) Animal Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other tertiary institution.

Signature……..

Date……..

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Abstract

In this study 240 commercial dairy goats (130 Saanen, 51 Toggenburg and 59 British Alpine) were genotyped with a panel of 25 microsatellite markers, 16 of which were on the FAO/ISAG recommended list for genetic diversity studies in *Capra hircus*. A moderate MNA of 8 was observed for all markers across all three breeds (ranging from 3 to 12 alleles per locus), and the mean PIC of the panel was 0.60. None of the loci investigated in this study were discarded due to HWE deviation, although some did deviate significantly from HWE within the breeds (5 in the Saanen, and 6 each in the Toggenburg and British Alpine). The overall diversity observed for the Saanen, Toggenburg and British Alpine were 62.6%, 63.4% and 63.4% respectively, indicating moderate diversity. Wright's F_{IS} values for the three breeds ranged between -0.063 to -0.005. Population structure analysis revealed six distinct populations, where the British Alpine and Toggenburg each formed individual clusters, and the Saanen formed three clusters. A crossbred population was also identified. Pedigree analysis found that most of the does contained in the herd book were culled before their third lactation. N_e ranges were estimated based on the available pedigree data of the Saanen (36–341), Toggenburg (18–63) and British Alpine (13–53). Average inbreeding values were 0.0632, 0.1335 and 0.0993 respectively. This study presents an insight to the genetic diversity of dairy goats in South Africa, and can be applied in the genetic management of the existing populations.

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Abbreviations

AMOVA	Analysis of molecular variance
AGR	Additive genetic relationship
AnGR	Animal Genetic Resources
BLUP	Best Linear Unbiased Prediction
B.C.	Before Christ
bp	Base pairs
DNA	Deoxyribonucleic acid
F	Inbreeding coefficient
Δ*F*	Inbreeding rate of change
FABI	Forestry and Biotechnology Institute
FAO	Food and Agriculture Organization of the United Nations
H_E	Expected heterozygosity
HGP	Human Genome Project
H_O	Observed heterozygosity
HWE	Hardy-Weinberg equilibrium
ISAG	International Society for Animal Genetics
MAF	Minor allele frequency
MNA	Mean number of alleles
n	Number
N_e	Effective population size
PCI	Pedigree completeness index
PCR	Polymerase chain reaction
PIC	Polymorphic information content
SAGS	Southern African Goat and Sheep Milk Processors Organization
SAMGBS	South African Milch Goat Breeders' Society
T_A	Annealing temperature
UN	United Nations
UP	University of Pretoria

Chapter 1

Introduction

1.1 Introduction

Livestock farming in South Africa is an important part of the food supply chain, as only 12% of the country's 1.2 million square kilometre surface area is suitable for crop farming activities (Department of Government Communciations and Information Systems, 2012). With a population that exceeds 50 million people, the importance of animal production efficiency is increasing, with the emphasis being placed on cost effective production while decreasing the impact on the environment (Sahlu & Goetsch, 2005). The modern domestic goat (*Capra hircus*) has historically been an efficient and adaptable provider of high quality meat and milk, as well as fibres and skins (Muller, 2005; Dubeuf & Boyazoglu, 2009). Currently it is estimated that the goat population in South Africa is around 6.2 million animals (FAO, 2012), of which 63% are unimproved indigenous types (Directorate: Animal Production, 2007).

The modern goat is believed to be a descendant of the Bezoar Ibex (*Capra aegagrus*) (Taberlet *et al.*, 2011). Archaeological evidence suggests that the Bezoar was originally domesticated in the area commonly known as the Fertile Crescent (Dubeuf & Boyazoglu, 2009; Taberlet *et al.*, 2011) – found along the borders of modern day Iran and Iraq (Boyazoglu *et al.*, 2005; Galal, 2005). Evidence of goat domestication was found in the Kermanshah Valley in Iran, dated to 8000 B.C. (Hatziminaoglou & Boyazoglu, 2004), although the analysis of mitochondrial DNA estimates that domestication occurred somewhat earlier, somewhere in the period between 9500 B.C. and 10500 B.C. (Naderi *et al.*, 2008). The goat was only the second domestication event in history, following that of the dog (*Canis familiaris*) around 11000 B.C. (Hatziminaoglou & Boyazoglu, 2004).

During the period from domestication until the present day the goat has spread across the globe. The reason for this is that the goat has proven to be eminently adaptable through its ability to utilize grazing as well as opportunistically feed on leaves and twigs (Alexandre & Mandonnet, 2005). This ability is aided by the goat's tolerance to high levels of condensed tannins (Waghorn, 2008). They have also been proven to thrive and produce when fed halophytic forage (Al-Shorepy *et al.*, 2010) and can tolerate extreme heat (Al-Tamimi *et al.*, 2013). This heat tolerance of goats is facilitated by their relatively small size which, along with their early maturity and frequent multiple kidding, has made the goat a popular choice for the smallholder and subsistence farmer (Alexandre & Mandonnet, 2005; Ahuya *et al.*, 2009). As a small ruminant able to use a broader range of forage, goats may be preferred above cattle, since more goats can be kept and cared for on an equivalent piece of land that may only be able to support a single bovine. The risk inherent with livestock keeping is also decreased, in the case where an animal may be lost to disease, predators or theft; the smallholder will still have a couple of goats left in his care, whereas he would have lost the only cow in his care, leaving him with nothing. The goat is also often seen as a form of "fluid

capital", where an animal could be more easily sold to cover immediate expenses, such as school fees or to purchase fodder for the rest of his herd (Peacock, 2005; Kosgey & Okeyo, 2007).

The Food and Agriculture Organization of the United Nations (FAO) estimated that the global goat population exceeded 970 million head in 2010 (FAO, 2012). It furthermore estimated that the largest number of goats can be found in Asia (60.1% of the world population), followed by Africa with 33.7%. The remaining 6.3% can be found in Europe, the Americas and Oceania. Goats tend to be more common in developing countries than in the developed countries (Alexandre & Mandonnet, 2005; Olivier *et al.*, 2005; Dubeuf, 2011), although some exceptions can be found.

Specialized dairy goats arrived in South Africa at the turn of the 20th century, originating from Switzerland and Britain. Originally four breeds were officially recognised in South Africa, namely the Saanen, Toggenburg, British Alpine and an Anglo-Nubian Swiss composite, although the Anglo-Nubian Swiss had disappeared by 1928 (Muller, 2005). There has been a tendency in South Africa to refer to dairy goats and their products as "milch" goats and "milch" goat products, because the remaining breeds were Swiss-type goats. In recognition that Swiss goat breeds are not the only specialized milk producing breeds in the world, throughout this dissertation the term "dairy" will be used instead of "milch" when referring to the type and character of the specialized milk producing breeds.

Today the dairy goat industry in South Africa supplies a niche market with specialty cheeses and fresh milk. The health benefits of goats' milk products both for those that are allergic to cows' milk products, as well as for the general populace, has led to an increase in the demand for these products and, as a result, an increased interest in keeping and breeding dairy goats (Olivier *et al.*, 2005). Goats' milk supply is however hampered by the seasonality of production seen in the commercial herds, where around 82% of the does kid in the spring (Muller, 2005), which results in a couple of months in a year when no fresh goats' milk is produced. The dairy goat population is also small, with less than 4000 registered animals, and currently does not produce enough to warrant investment in large scale freezing facilities to ensure year-round supply (Directorate: Animal Production, 2007).

The commercial dairy goat population – consisting mostly of Saanen, Toggenburg and British Alpine animals - in South Africa originates from only a limited number of foundation animals that was imported at the start of the 20th century. Despite some limited additional imports that have been made throughout the succeeding years, the South African population have been isolated from the rest of the world's goat production centres largely due to logistical difficulties. With the increased interest in keeping dairy goats, concerned breeders have questioned whether there is enough variation within the commercial population to support the growing industry.

1.2 Aim of the study

The South African dairy goat breeds have never been characterised with molecular genetic techniques. Several other small stock breeds used for commercial production in South Africa, such as the SA Boer,

Savannah and Kalahari Red goats (Pieters *et al.*, 2009), Angora goats (Visser *et al.*, 2010) as well as Karakul and SA Mutton Merino sheep (Buduram, 2004) have been characterized in order to improve the genetic management of these breeds. A quantitative study by Muller (2005) was conducted to determine genetic parameters for the production traits of the commercial dairy goat populations. He found however that the number of records for the Toggenburg and British Alpine populations were too few to perform a statistically significant estimation of genetic parameters for these breeds. Although the estimations done for the Saanen breed were consistent with results obtained in similar studies, the results were considered uncertain, as only a portion of the records were complete enough to use in the evaluation.

The aim of this study was to assess the genetic diversity of the South African commercial dairy goat population, using a panel of microsatellite markers selected from the panel recommended by the International Society of Animal Genetics (ISAG), with additional markers included from similar studies. The population structures for the three breeds were also investigated using the available pedigree records to estimate inbreeding and herd structures. The effective population sizes of these breeds were also determined.

Three major commercial breeds, namely the Saanen, the Toggenburg and the British Alpine, were included in this study. At least 50 unrelated animals per breed were included, according to the FAO guidelines for studies on genetic diversity in small populations. Animals that were sampled were from commercial farms in the provinces of the Western Cape, Northern Cape, KwaZulu Natal, Limpopo, Northwest, Free State and Gauteng.

Chapter 2

Literature Review

2.1 Introduction

The dairy goat population in South Africa makes up only a small part of the total estimated goat population for the country. The three breeds used most commonly by commercial producers are the Saanen, Toggenburg and the British Alpine, all three of which are considered as "international breeds" as they are found and used in several different countries. The South African dairy goat industry is small in comparison to some of the developed countries such as France and Spain, and supplies a niche market with speciality cheeses. An increase in local demand for goats' milk products has led to a simultaneous increase in the interest in keeping dairy goats, and concerned breeders have questioned whether these isolated populations have enough genetic diversity to support the growing industry.

In this review a brief overview of dairy goat production on a global basis will be given, and the South African breeds and population management will then be discussed. There will also be a case made for the use of molecular markers to determine the genetic diversity of a population.

2.2 Dairy goat production on a global basis

The Food and Agriculture Organization of the United Nations (FAO) estimated that the global goat population exceeded 970 million head in 2010 (FAO, 2012). Between 1990 and 2010, the global goat population increased by 65%, and the population in Africa increased by 84%. In contrast, the European goat population decreased by 23% during the same time period. The population in South Africa has remained relatively constant (Directorate: Animal Production, 2007). In Table 2.1 it can be seen that the top five countries in terms of their goat populations are all developing countries. The South African production figures are included for comparison.

Table 2.1 Top five global countries in terms of goat numbers, and their products (measured in tonnes) compared to that of the South African goat population for the year 2010

Country	Goat numbers	Fresh goat milk (tonnes)	Goat meat (tonnes)	Goatskins (tonnes)
China	195 855 554	277 228	1 921 854	390 287
India	154 000 000	4 594 000	586 500	160 020
Pakistan	59 858 000	739 000	278 000	99 162
Nigeria	56 524 075	No data	287 655	45 300
Bangladesh	51 400 000	2 496 000	191 100	73 400
South Africa	**6 274 846**	**No data**	**35 480**	**600**

Source: FAOSTAT, 2012

In developed countries, goats and goat products became somewhat side-lined in the face of the rapid development of high capacity dairy cattle and increasing urbanization. This is also partly due to the historical bias against the goat as the "poor man's cow" as well as its destructive feeding habits (Boyazoglu *et al.*, 2005; Dubeuf & Boyazoglu, 2009). The changing lifestyle in the developed world has reawakened interest in the goat however. The trend towards healthier eating habits, animal welfare concerns and the environmental impact of production has caused consumers to be more open to products from less "traditional" animals (Grunert, 2006; Barillet, 2007).

The development of dairy goats in France is of particular interest. French dairy goats consist mainly of Saanen and Alpine breeds, and producers tend to favour intensive systems for production (Danchin-Burge *et al.*, 2012). Recording of these goats began in the 1960's, and goats were first selected based on protein yield; conformation traits, fat and protein content were later added to the selection criteria. All breeding animals have been evaluated with the BLUP animal model since 1992, and breeding values are available for all animals. The result of such an organized breeding programme is that the genetic progress for protein yield, fat content and milk yield has been positive for both the Saanen and the Alpine between 1990 and 2010 (Danchin-Burge *et al.*, 2012). Although the French are not the largest goat milk producer globally, it can be seen in Table 2.2 that the average milk yield per goat, even when calculated empirically, is much higher than the competing countries.

Table 2.2 Top ten countries in terms of goat milk production in 2010, in comparison to South African production estimates

Country	**Production (tonnes)**	**Goat population**	**Average kg per goat[1]**
India	4 594 000	154 000 000	29.83
Bangladesh	2 496 000	51 400 000	48.56
Sudan (former)	1 512 000	43 441 000	34.81
Pakistan	739 000	59 858 000	12.35
Mali	689 234	16 522 454	41.71
France	648 436	1 434 511	452.03
Spain	507 000	2 933 800	172.81
Somalia	500 600	11 500 000	43.53
Greece	405 800	4 850 000	83.67
Iran (Islamic Republic of)	306 000	23 000 000	13.30
South Africa	**1 400[2]**	**6 274 846**	**0.22**

Source: FAOSTAT, 2012

[1] Empirical calculation based on all goats in population

[2] Unofficial estimates (Directorate: Animal Production, 2007)

Goat's milk in France is mainly used for the production of high quality cheeses (Danchin-Burge *et al.*, 2012). France is the second largest producer of goat cheese globally (Table 2.3), and the average yield per goat far outstrips the production compared to the rest of the top ten countries. The production of goat's milk and goat's cheese were shown in Table 2.2 and Table 2.3, and highlights the importance of these products in the developing countries, such as India and the former Sudan (Lopes *et al.*, 2012), where goats in small-holder systems are becoming more important to supply animal products.

Table 2.3 Top ten countries in terms of goat cheese production in 2010

Country	Production (tonnes)	Goat numbers	Average cheese kg per goat[1]
Sudan (former)	110 000	43 441 000	2.53
France	95 717	1 434 511	66.72
Greece	48 000	4 850 000	9.90
Spain	41 160	2 933 800	14.03
Iran (Islamic Republic of)	38 327	23 000 000	1.67
Niger	31 011	13 673 073	2.27
Mexico	16 700	8 993 221	1.86
Tajikistan	16 440	1 582 811	10.39
Afghanistan	10 080	6 789 000	1.48
China	7 800	195 650 000	0.04

Source: FAOSTAT, 2012 [1] Empirical calculation based on all goats in population

Another example of a country where interest in goat milk products are growing is Canada. The production of goat milk in Canada was estimated at 21 million litres in 2004 (Agriculture and Agri-Food Canada, 2006), a large part of which is also used for the production of cheeses. The Canadian Goat Society (http://goat.softcorp.ca) and the Canadian National Goat Federation (http://www.cangoats.com/) represents the fibre, meat and dairy goat producers in Canada. These bodies administer goat registrations; promote information regarding herd health and disease threats, and offers milk testing programs.

2.3 Dairy goats in South Africa

Goat population and distribution

The FAO estimates that the South African goat population consists of about 6.2 million animals (FAO, 2012). This estimate is complicated by the fact that most of the goats in South Africa (63% as estimated by the Department of Agriculture, Forestry and Fisheries Directorate: Marketing (2012)) consists of unimproved indigenous goats in the non-commercialized agricultural sector (Department of Government Communciations and Information Systems, 2012). These goats do not participate in a recording scheme, and without official statistics exact numbers are difficult to determine (Directorate: Animal Production, 2007). The majority of the South African goat population is found in the Eastern Cape (Figure 2.1), followed by

Limpopo and KwaZulu Natal. The Eastern Cape is also home to an estimated 910 000 Angora goats (Directorate: Marketing, 2010) which supplies more than 50% of the global mohair clip (Visser *et al.*, 2011a). The remaining 1 384 000 goats are mainly the improved meat goat breeds, namely the Boer, Savannah and the Kalahari Red. The commercial dairy goats are in the minority, with less than 4 000 goats registered with South African Stud Book (SA Stud Book, PO Box 270, Bloemfontein, 9300).

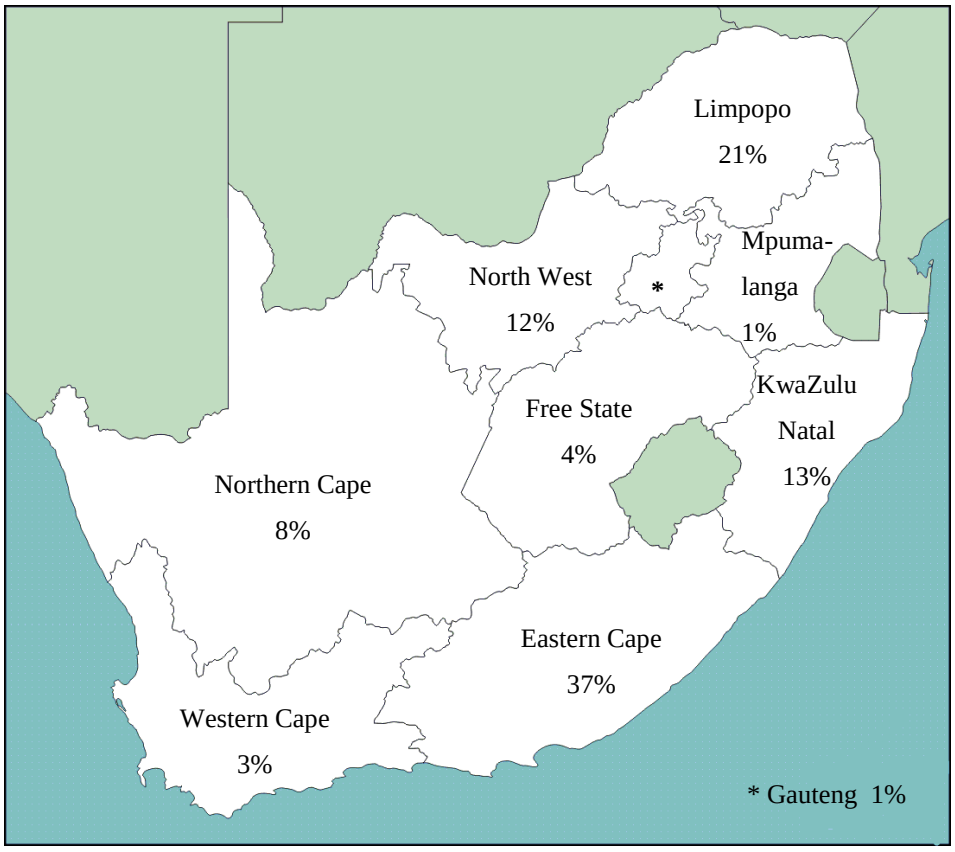


Figure 2.1 Map of South Africa showing the distribution of the South African goat population in each of the provinces

Commercial dairy goats are mainly distributed around the economic centres in Gauteng and the Western Cape, where goats' milk products are more easily marketed. Producers are also found in the Northern Cape, Eastern Cape, Free State, KwaZulu Natal and Limpopo.

Commercial dairy goats in South Africa generally belong to one of three breeds, namely the Saanen, the Toggenburg and the British Alpine. There is also a very small population (less than 40) of registered Bunte Deutsche Edelziege (BDE) goats, but these animals are found on less than five farms. These exotic breeds are preferred for commercial milk production, despite being vastly outnumbered by the indigenous goat population, because of their increased production levels and the predictability of production. Donkin & Boyazgolu (2000) found that indigenous goats produce 23kg of milk over a lactation spanning 93 days, which is much less in comparison to the exotic dairy breeds. Table 2.4 indicates that the Saanen, Toggenburg and British Alpine does produced on average more than 1 200kg milk during the 2012/2013 lactation period.

The average days in milk were also two to three times the length of the lactation period recorded for the indigenous goats in the study by Donkin & Boyazgolu (2000).

Table 2.4 Lactation statistics for the British Alpine, Saanen and Toggenburg for the 2012/2013 period

Breed	**Lactations recorded**	**Average milk (kg)**	**Average protein %**	**Average fat %**	**Average days in milk**
British Alpine	45	1366	3.6	5.0	202
Saanen	85	1227	3.2	4.0	317
Toggenburg	30	1297	3.6	4.9	215

Source: SA Stud Book

The Saanen goat originated in the Saanen Valley in Switzerland, (Glowatzki-Mullis *et al.*, 2008; Gurung & Solaiman, 2010) and is characterized as a medium to large goat with a white coat, and may either have sabre-shaped horns or be naturally polled. The Saanen is also well-known for larger milk volume production, although the butterfat levels are average. It is one of the most widely distributed dairy goat breeds, being found in more than 68 countries (Gurung & Solaiman, 2010). The Swiss Saanen herdbook was established in 1890, and Saanen goats were first imported to South Africa in 1898 (Olivier *et al.*, 2005). The Saanen comprises the largest number of dairy goats in South Africa, according to the SA Milch Goat Breeder's Society (2013). Due to its light pigmentation, the Saanen is susceptible to sunburn in South African conditions (Muller, 2005).

Figure 2.2 Saanen doe (photo by J.J. Bosman)

The Toggenburg is also a Swiss breed, originating in the Toggenburg Valley in the north-east of Switzerland (Gurung & Solaiman, 2010). This goat has typical Swiss markings – which consists of white stripes down the face coupled with a light belly and legs (Adalsteinsson *et al.*, 1994) – on a brown coat. The Toggenburg was imported to South Africa in the early 20th century (Hofmeyr, 1968). It is a medium sized

goat, and produces milk with a higher butterfat content than the Saanen (Table 2.4). Its darker pigmentation gives it an advantage over the Saanen as far as sunburn is concerned.

Figure 2.3 Toggenburg doe (photo by J.J. Bosman)

The British Alpine is a black goat with typical Swiss markings, and was developed from various Alpine-type goats of French and Swiss origin that were imported to Great Britain in 1903 (Gurung & Solaiman, 2010). This composite breed (Shrestha, 2005) is a medium to large frame animal (Hofmeyr, 1968), and in 1925 the British herdbook was established after gaining recognition in 1921 (Gurung & Solaiman, 2010). The British Alpine was imported to South Africa between 1924 and 1934, according to the South African Milch Goat Breeders' Society (SAMGBS). The British Alpine had the highest average butterfat recorded during the 2012/2013 lactation period (Table 2.4), and also had the highest average milk production of all three breeds, averaging 1 366kg of milk per doe.

Figure 2.4 British Alpine doe (photo by the SA Milch Goat Breeders' Society)

Registration data obtained from SA Stud Book indicate that, although the dairy goat industry in South Africa is small and serves a niche market, there is a growing interest in keeping and breeding dairy goats (Muller, 2005). The number of animals registered between 1990 and 2012, according to their year of birth are given in Figure 2.5 (Saanen) and Figure 2.6 (British Alpine and Toggenburg). The Saanen is the most

popular breed, and 488 of the kids born in 2012 were registered. It is also worth noting that registrations remained low during the 1990's, with less than 60 animals registered per year; however, registrations have increased dramatically from 2004 onwards.

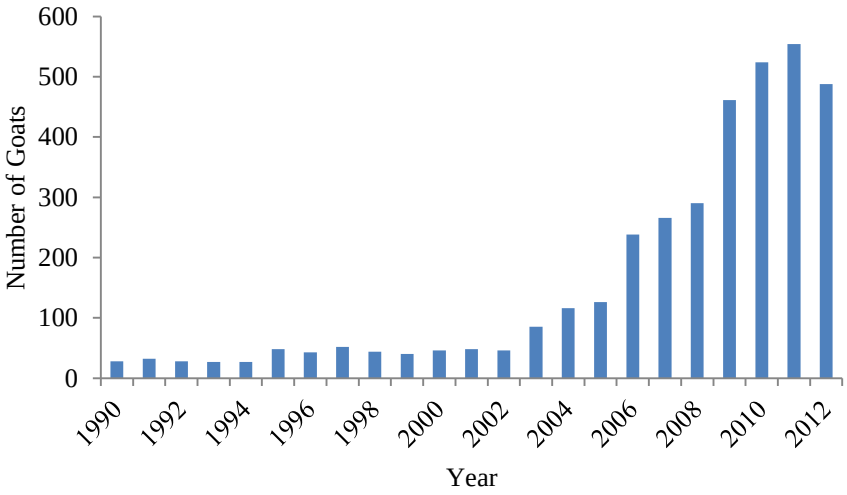


Figure 2.5 Registrations of Saanen goats by year of birth (1990-2012) Source: SA Stud Book

The British Alpine and the Toggenburg are much fewer in number in comparison with the Saanen (Figure 2.6), but a similar trend can be seen, with very low numbers of registrations being recorded in the 1990's, and a more gradual rise in registrations from 2000 onwards. The popularity of both breeds varies from year to year; in 2010 74 Toggenburg kids were registered in comparison to 64 British Alpine kids, while in 2012 much more British Alpine kids were registered than Toggenburg kids (78 versus 22).

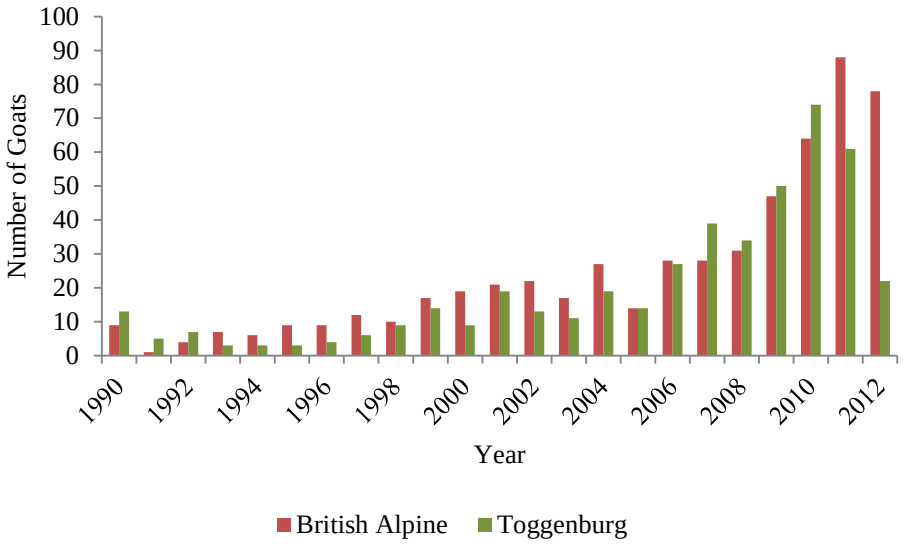


Figure 2.6 Registrations of British Alpine and Toggenburg goats by year of birth (1990-2012) Source: SA Stud Book

The dairy goat industry supplies a niche market in South Africa. Dairy goat products are often used as a suitable replacement for cow's milk, where infants and even adults display allergic reactions to the aforementioned (Haenlein, 2004). Many South African producers focus on the manufacturing of cheeses and other value-added products. Marketing of these products occur mostly in an informal way, such as by selling directly to consumers via on-farm sales, or at fresh food, organic or farmer's markets held over weekends. Limited quantities of local goat's milk products are sold through retailers and supermarket chains, and it is therefore difficult to estimate the true volumes of milk that is produced. Unfortunately, goat's milk production is also highly seasonal in South Africa. Out-of season demands are satisfied by importing powdered goat's milk (Directorate: Animal Production, 2007). Due to the informal trade in South Africa, no official milk production figures exist, but unofficial estimates gauge the South African goat milk production around 1.4 million tonnes per annum (Directorate: Animal Production, 2007).

Population management

The SAMGBS remarks that for these three breeds, the original imported animals were most likely not kept pure and were probably bred to other dairy-type goats (Muller, 2005). The location of South Africa is such that genetic material cannot be readily exchanged with other major centres of dairy goat production due to the distances and other logistical issues, including outbreaks of diseases such as Foot-and-Mouth, and the prevalence of endemic diseases. South Africa is free of scrapie (Directorate: Agricultural Information Services, 2003), and therefore importing live goats from areas where scrapie is endemic is prohibited. By keeping limited imported stock pure, the risk is being taken that the gene pool becomes too small through inbreeding. Despite the importation of pure stock from time to time (Muller, 2005), the loss of genetic diversity is a very real threat due to the relatively small population sizes. The issue of conserving genetic

diversity in small populations is a frequent research theme, as evidenced by Kumar *et al.* (2005), Glowatzki-Mullis *et al.* (2008), Taberlet *et al.* (2011) and Dixit *et al.* (2012).

Table 2.5 Heritability estimates and standard errors for production traits obtained for the South African Saanen using 1st and 2nd parity records of stud animals, as well as records over all parities from a commercial herd (Muller, 2005)

Trait	1st Parity	2nd Parity	Commercial herd
Milk yield	0.32 ± 0.08	0.20 ± 0.10	0.31 ± 0.06
Fat yield	0.37 ± 0.08	0.18 ± 0.11	0.21 ± 0.05
Protein yield	0.31 ± 0.08	0.24 ± 0.10	0.31 ± 0.06
Fat %	0.67 ± 0.08	0.34 ± 0.12	0.12 ± 0.05
Protein %	0.32 ± 0.08	0.24 ± 0.11	0.28 ± 0.07

A quantitative study by Muller (2005) was conducted to determine genetic parameters and heritabilities of the production traits in the commercial dairy goat populations. It was determined that the numbers of Toggenburg and British Alpine records were insufficient to determine statistically significant heritability estimations, and were therefore excluded from the study. The results obtained for the Saanen breed (Table 2.5) were consistent with results obtained in similar studies (Morris *et al.*, 1997; Montaldo & Manfredi, 2002; Torres-Vázquez *et al.*, 2009).

There are no more than a couple of producers in South Africa that produce dairy goat products on a true commercial scale; the largest portion of the dairy goat population is found on these intensive production systems (Muller, 2005). The rest of the producers in South Africa keep commercial dairy goats in a small-holder setting, with herds rarely exceeding 100 animals in number. Producers are furthermore divided between those that breed stud animals - represented by the South African Milch Goat Breeders' Society (www.milkgoats.co.za /milkgoat_society/) - and producers that wish to breed for the sake of production, and not necessarily breed stud animals. These producers are represented by the Southern African Goat and Sheep Milk Processors Organization (SAGS) (www.milkgoats.co.za/milkgoat_production/). SAGS also certifies the goat's milk products produced by its members, provided that the goats it was produced from are at least 7/8 Swiss-type dairy goats. This accommodates commercial farmers who make use of cross-breeding practices to improve the butterfat content of the milk, usually by crossing Saanen with Toggenburg. The F_1-generation is however crossed back to one of the parent breeds, or to a third breed, such as the British Alpine. It should be noted that the commercial production situation in South Africa is similar to the scenario as described by Dýrmundsson (2006); dairy goats and their products are not normally the primary source of income for their producer, but rather an expansion on other farming activities, or even completely unrelated to the producer's primary source of income. According to Muller (2005) a large part of the dairy goat industry in South Africa can be described as a "hobby" industry.

Most of the registered goats are stud animals, which should be taken into consideration when trying to estimate the total number of dairy goats in South Africa. Commercial animals used purely for production purposes will generally not be registered (Muller, 2005), and recording of pedigrees among commercial farmers are frequently poor to non-existent. This is partly due to the fact that most commercial farmers make use of group mating and over-mating, and therefore the specific sire of the progeny cannot be determined. Goat bucks are also nimble escape artists, and will frequently jump fences from one breeding group into another, thereby further complicating pedigree recording.

Dairy goats are also included in the National Dairy Improvement Scheme along with dairy cattle (Olivier *et al.*, 2005). The official milk recording scheme requires that the total daily milk production is recorded over the lactation period. Every five weeks a milk sample is also taken and analysed for fat, protein and lactose proportions. Eight such tests over the lactation is required to complete a lactation record (Muller, 2005). Participation by dairy goat producers is poor however, with less than 100 animals across all breeds participating during the first six months of the 2012/2013 season (personal communication, Dr BE Mostert, bernice@studbook.co.za, 2013). There are several misperceptions among various dairy goat producers regarding the participation in the official milk recording scheme, such as that the process is difficult and expensive (Muller, 2005), and that it holds no tangible benefit for them.

It has been difficult to estimate the population status of the Saanen, Toggenburg and British Alpine breeds in South Africa due to the lack of complete data. The lack of pedigree data complicates the estimation of the genetic diversity of these breeds, and therefore the estimation and monitoring of inbreeding levels and the effective population size cannot be done with any accuracy. Monitoring these parameters are important in these small populations to prevent the loss of diversity, which would impact the ability of these breeds to survive a population disaster (Bodó, 1989), or ability to respond to selection pressure and make genetic progress in production traits.

2.4 DNA markers used to quantify genetic diversity

The advances in computing power and technology has contributed significantly to the advances in genetic technology (Bourdon, 2000; Boettcher, 2001). The Human Genome Project (HGP) officially started on 1 October 1990 (Falcón de Vargas, 2002), and was only completed in 2001. In 2003, a Hereford cow was used to sequence the bovine genome (Burt, 2009), and the sequencing project was completed only six years later, in 2009. This apparent acceleration in completing whole-genome sequencing was due to the increase in computing power, as well as the application of technology developed during the HGP, such as the DNA microarray, and the decrease in costs to complete such projects (Falcón de Vargas, 2002). These developments have also benefitted the goat, as its genome was mapped and sequenced in 2012 (Dong *et al.*, 2013) using a female Yunnan goat. Before this, the goat genetic map was relatively undeveloped, especially

in comparison with the cattle and sheep map, although the goat map had good agreement with the ovine linkage map (Maddox & Cockett, 2007).

Before the development of DNA-based markers, both animal and plant breeding relied on morphological and biochemical markers to estimate the likely genotypes of the target organism (Collard *et al.*, 2005). DNA-based markers, in the form of restriction fragment length polymorphisms (RFLP's), were developed for use in forensic investigations in humans by 1984 (Dodgson *et al.*, 1997; Tamaki & Jeffreys, 2005). The advantage in using DNA-based markers lies in being more abundant than either the biochemical or morphological markers. Environmental conditions or the developmental stage of the organism are furthermore unlikely to affect the markers (Collard *et al.*, 2005).

Microsatellites consist of tandem repeats between two and six base pairs long (Beuzen *et al.*, 2000; Bhargava & Fuentes, 2010), and can be abbreviated as STR's (short tandem repeats). Repeats larger than six base pairs are termed minisatellites, or variable number tandem repeats (VNTR), and can be up to 100 base pairs in size (Dodgson *et al.*, 1997). Microsatellites are also co-dominant, highly polymorphic with a high polymorphic information content (PIC) (Dodgson *et al.*, 1997). Microsatellites often occur in the non-coding regions, which means that mutations outside a recognition site or the coding region has a reasonable chance of being identified (Beuzen *et al.*, 2000). The length of a microsatellite with a certain repeat, e.g. $(CA)_n$, distinguishes the different alleles (Bhargava & Fuentes, 2010). In this example, $(CA)_4$ and $(CA)_6$ will be two different alleles.

Repeat number and repeat type affects the stability of a microsatellite; longer repeats, such as pentanucloetides and hexanucleotides, have a greater chance of mutation by substitution, while dinucleotide repeats may experience slippage of the DNA polymerase, causing the lengthening or shortening of the microsatellite (Ellegren, 2004; Buschiazzo & Gemmell, 2006; Bhargava & Fuentes, 2010). The class of microsatellites that is most useful in population studies are the dinucleotides, as they are generally evenly distributed throughout the genome; in animals these microsatellites tend to consist mostly of $(CA)_n$ repeats (Jarne & Lagoda, 1996; Beuzen *et al.*, 2000; Bhargava & Fuentes, 2010). Trinucleotide and tetranucleotide microsatellites occur less often throughout the genome, and tend to cluster in certain regions - such as around the centromeres in the case of tetranucleotide microsatellites (Jarne & Lagoda, 1996; Ellegren, 2004).

Table 2.6 Genetic diversity studies performed on various goat breeds using microsatellite markers

Study title	n Markers	Authors
Genetic diversity in Swiss goat breeds based on microsatellite analysis	20	Saitbekova *et al.* (1999)
Genetic diversity of Southern Italian goat populations assessed by microsatellite markers	15	Iamartino *et al.* (2005)
Population structure, genetic variation and management of Marwari goats	25	Kumar *et al.* (2005)
DNA microsatellites to ascertain pedigree-recorded information in a selecting nucleus of Murciano-Granadina dairy goats	9	Jiménez-Gamero *et al.* (2006)
Analysis of the genetic structure of the Canary goat populations using microsatellites	27	Martínez *et al.* (2006)
Genetic diversity measures of Swiss goat breeds as decision-making support for conservation policy.	47	Glowatzki-Mullis *et al.* (2008)
Genetic diversity of five Chinese goat breeds assessed by microsatellite markers	11	Li *et al.* (2008)
Genetic diversity and relationships of 10 Chinese goat breeds in the Middle and Western China	20	Qi *et al.* (2009)
South African developed meat type goats: a forgotten animal genetic resource?	19	Pieters *et al.* (2009)
Genetic characterisation of Burkina Faso goats using microsatellite polymorphism	27	Traoré *et al.* (2009)
Genetic diversity and population structure in Portuguese goat breeds.	25	Bruno-de-Sousa *et al.* (2011)
Genetic diversity and relationship among Indian goat breeds based on microsatellite markers	25	Dixit *et al.* (2012)

n: number

Due to the variation in repeat length, microsatellites are usually identified by a primer in the flanking region, often tagged fluorescently or radioactively (Beuzen *et al.*, 2000; Zane *et al.*, 2002). It may therefore occur that a point mutation in the flanking region will leave a primer unusable, and the particular allele cannot be identified. Detection of null alleles can be done during population studies by testing the observed allele frequencies against the frequencies expected when a population is in Hardy-Weinberg equilibrium (HWE).

Microsatellites are preferred for population studies (Baumung *et al.*, 2004; Morin *et al.*, 2004; FAO, 2011), especially when characterizing a population for the first time. The cost associated with the discovery

of new microsatellites is high (Bhargava & Fuentes, 2010) due to the sequencing requirements. Once discovered though, microsatellites have the advantage of having highly conserved flanking sequences, which allows the microsatellites to be used across species (Jarne & Lagoda, 1996; Kim *et al.*, 2004). The eventual cost of microsatellites therefore decreases, as discovery and sequencing of microsatellites does not need to be done separately for every species. Microsatellites are also easily used and the results are reproducible (Bhargava & Fuentes, 2010). The popularity of microsatellites in population studies also allows the results from the different studies to be compared with each other (Baumung *et al.*, 2004), such as in Table 2.6.

2.5 Quantifying genetic diversity

There are a number of goat breeds in the world today, of which 136 have been clearly defined (Dubeuf & Boyazoglu, 2009), although Galal (2005) estimated that there are 570 types of goat world-wide. The concept of breed only crystalized in the 18th century (Boyazoglu *et al.*, 2005) when economically-driven selection of farm animals, such as the dairy-type goat, began (Dubeuf & Boyazoglu, 2009). The development of high-producing breeds has highlighted the differences between breeds (FAO, 2011), but at the same time eroded the diversity within breeds, as animals with similar characteristics were often used as the founder populations (Toro *et al.*, 2009; FAO, 2011).

Table 2.7 Threshold numbers to determine the threat status of domestic populations (Bodó, 1989)

Status	**Number of breeding females**	**Description**
Extinct	<0	No possibility of restoring the population, no purebred males or females can be found
Critical	<100	Close to extinction, genetic variability reduced to below that of the ancestral population, action to increase the population size is essential if it is to survive
Endangered	100 – 1000	In danger of extinction because the effective population size (N_e) is too small to prevent genetic loss through inbreeding, which will result in a reduction in the viability of the breed
Insecure	1000 – 5000	Population numbers decreasing rapidly
Vulnerable	5000 – 10000	Some disadvantageous effects endanger the existence of the population, and some precautionary measures should be taken to prevent further decline
Normal	>10000	Population not in danger of extinction; can reproduce without genetic loss; no visible changes in population size.

Selection for specific production traits within a breed decreases within-breed diversity even further, and between-breed diversity is also decreased, as several breeds are selected for similar production traits. This, along with practices such as inbreeding and line-breeding, leads to individuals within a breed or

population to become genetically indistinct from each other, resulting in a lower effective population size (N_e). Populations or breeds with a small N_e run the risk of being unable to recover from events such as epidemics or other disasters, and may run the risk of becoming extinct, as indicated by the FAO guidelines set forth in Table 2.7. By quantifying the N_e in a seemingly healthy population from time to time, genetic drift within the population can monitored and managed to decrease the loss of diversity (Toro *et al.*, 2009).

The global distribution of the Saanen, the Toggenburg and the British Alpine is such that none of these three breeds are likely to become extinct anytime soon. The situation in South Africa though, considering the difficulties associated with importing new stock and keeping the breeds pure, has probably had an impact on the diversity seen within the local populations. Additionally, erratic recordkeeping by producers result in animals with incomplete pedigrees, and the practice of interlending bucks between farms increases the risk of inadvertent inbreeding in the South African populations.

Genetic diversity can be quantified in several ways when using molecular markers. Genotypic and allelic frequencies is one measure, while the polymorphicity of the markers used in a study is another (Toro *et al.*, 2009). A large number of alleles at a specific locus, of which the minor allele frequencies (MAF) are above 0.05, are preferable when selecting markers for use in such a study (Nei, 1987), as it is implied that genetic variation is increased when a large number of alleles are present. Allelic frequencies are also more sensitive to historical population bottlenecks than expected heterozygosity (H_E) (Toro *et al.*, 2009), and reflects changes in population sizes more accurately.

Judging genetic diversity by the allelic frequencies alone could skew results however, by giving more weight to rare alleles (Falconer, 1989). The average unbiased expected and observed heterozygosities (H_E and H_O) over all the loci used in a study (Nei, 1978; Falconer, 1989) is another method to determine the genetic diversity of a population. H_E tests for the expected average frequency of heterozygotes over all the loci used in a diversity study, with little influence from rare alleles. H_E is then compared to H_O in order to identify significant deviations between the expected and observed heterozygote frequencies. A decrease in the average observed heterozygosity would be an indication of a population that experienced either a recent bottleneck or that is under intensive selection, which would result in a loss of diversity.

The partitioning of genetic diversity within breeds and between breeds can be visualized by conducting an analysis of molecular variance (AMOVA). The AMOVA tests the variation observed in the population, and partitions such variation accordingly. Such variation may be due to the variation seen between breeds, the variation of individuals within a breed or due to the variation seen among the individuals themselves (Excoffier & Lischer, 2010). The partitioning of these variance components also gives some insight into the population structure of the breeds included in the AMOVA.

The structure of the populations under investigation can be further determined with Wright's *F*-statistics (Weir & Cockerham, 1984; Falconer, 1989). Several fixation indices are generated that utilizes the inbreeding coefficient (*F*). F_{IS} compares the inbreeding coefficient of an individual to that of its specific subpopulation, while F_{ST} compares the subpopulation to the total population. F_{IT} considers the inbreeding

coefficient of the individual relative to the total population (Falconer, 1989). These fixation indices are used to determine the loss of heterozygosity within a population.

The population structure can be visualized graphically by using genetic computer software such as STRUCTURE, developed by Pritchard *et al.* (2000). STRUCTURE is used to determine the true number of populations (K), by using Bayesian-based assignment principles. The software ignores available population information, therefore identifying distinct genetic populations, and assigns individuals based upon their genetic membership. The model used for the simulation assumes admixture in the ancestry, and therefore assumes correlated allele frequencies. The distinct genetic populations identified can then be graphically presented as clusters, which can then be manipulated to determine whether or not animals included in the study clusters with their own breed. From the STRUCTURE results it is also possible to determine whether there is any introgression or cross breeding present when comparing one population to another.

Pedigree data can be used to determine population structure (Groeneveld *et al.*, 2010), although in order to obtain highly accurate results, the pedigrees in the herd books should be as complete as possible. From pedigree data it is possible to determine the generation interval of a population, and compare it to the generation interval that may be observed along specific selection pathways, such as the sire-to-son, sire-to-daughter, dam-to-son or dam-to-daughter selection pathways. Family sizes and influential ancestors can also be identified. The effective population size (N_e) can be estimated from the population structure (Villanueva *et al.*, 2010), which can then be used to determine the threat status of a population, as shown in Table 2.7. FAO guidelines suggest that an N_e of at least 50 animals should be maintained (FAO, 1998). Additional parameters such as the average inbreeding coefficient (F) and the rate of change of the inbreeding coefficient (ΔF) can be used to manage the genetic drift in a population. In a small population the inbreeding coefficient is not sufficient to describe a population, and the rate of inbreeding change is more accurate (FAO, 1998); the ΔF should not exceed 1% per generation.

Herd life is an important parameter in a dairy goat herd, as the first lactation production of the dairy goat, similar to the dairy cow, is typically lower than that seen in later lactations (Muller, 2005; Goetsch *et al.*, 2011). For the dairy goat to be an efficient producer, she needs to stay in the herd until at least the third or fourth lactation, when peak production is attained (Goetsch *et al.*, 2011). Parity records will therefore give an indication whether or not the dairy goat population is performing according to their potential by giving an insight into the erosion rates observed in the dairy herd.

2.6 Conclusion

The South African dairy goat breeds used for the commercial production of goats' milk products, namely the Saanen, Toggenburg and British Alpine, have largely been isolated from the dairy goat production centres in the rest of the world. While the industry in South Africa is small in comparison to those in more developed countries, there has been an increased interest in keeping dairy goats due to the increasing demand for goats' milk products. Due to the isolation of these breeds, concerns have been raised that there

may not be enough genetic variation within the population to support the growing industry. Recordkeeping in the small stock sector in South Africa is generally poor, and the lack of parentage and production data from the general commercial population has made results obtained from quantitative studies uncertain. Determining the genetic diversity through molecular markers has been successful in several studies, and has the advantage of producing significant results despite prior population information being incomplete.

Chapter 3

Materials and Methods

3.1 Introduction

The aim of this study was to determine the genetic diversity of three commercial dairy goat breeds in South Africa – namely the Saanen, the Toggenburg and the British Alpine. This was done by collecting blood samples from a total 240 animals and genotyping the samples with 25 microsatellite markers. Ethical approval (EC088-12) for the study was obtained from the University of Pretoria Animal Use and Care Committee in the Faculty of Natural and Agricultural Sciences prior to the commencement of the project.

3.2 Population sampling and breeder survey

Blood samples were collected from 240 dairy goats, representing 130 Saanen, 51 Toggenburg and 59 British Alpine goats. Animals were sourced from commercial dairy goat farms in the Western Cape, Northern Cape, Gauteng, KwaZulu Natal, Limpopo, the Free State and North West provinces of South Africa (Figure 3.1).

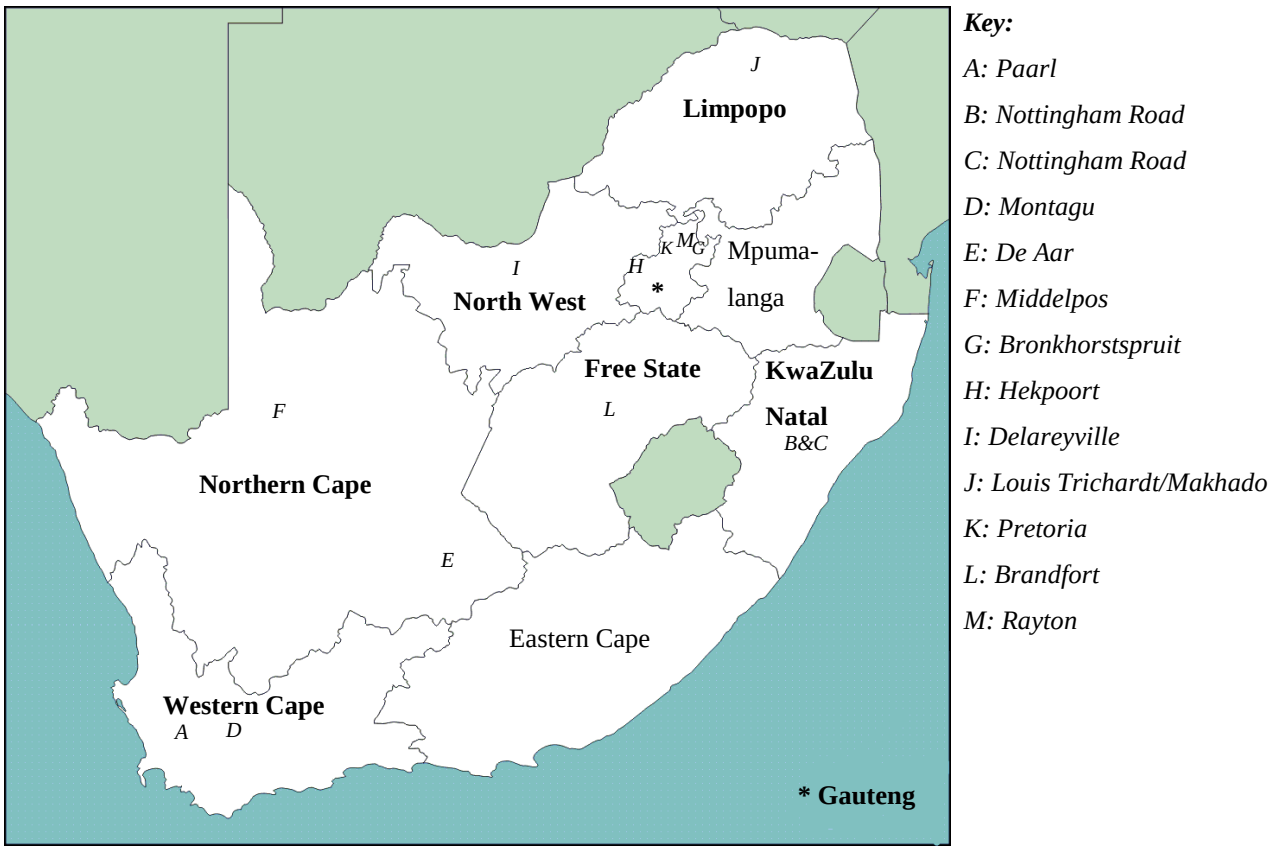


Figure 3.1 Sampling locations with the sampled provinces printed in bold, and the closest town indicated by italics

Several of these farms represent stud breeders, and had complete pedigree records available, which made the task of sampling unrelated animals easier. In the case of incomplete or unavailable pedigree records, animals were randomly sampled based on difference in age and production status, in order to increase the probability of sampling unrelated animals. The distribution and origin of the goats sampled are shown in Table 3.1. It should be noted that while farms E and F are treated separately, these animals were all sampled at farm D. The reason for this is that farm D only recently started breeding dairy goats, and the animals attributed to farms E and F are animals that were sourced from these farms as the foundation stock.

Table 3.1 Origin, distribution and number of the Saanen, Toggenburg and British Alpine goats sampled

Breed	**Farm**	**Province**	**♀ Sampled**	**♂ Sampled**	**Total samples**
Saanen	A	Western Cape	38	3	41
	B	KwaZulu Natal	4	1	5
	C	KwaZulu Natal	9	1	10
	D	Western Cape	3	0	3
	E	Northern Cape	9	0	9
	F	Northern Cape	10	0	10
	H	Gauteng	5	0	5
	J	Limpopo	21	5	26
	K	Gauteng	10	6	16
	L	Free State	1	0	1
	M	Gauteng	4	0	4
Toggenburg	A	Western Cape	6	0	6
	B	KwaZulu Natal	5	1	6
	G	Gauteng	9	5	14
	H	Gauteng	5	0	5
	I	North West	9	1	10
	L	Free State	10	0	10
British Alpine	A	Western Cape	14	1	15
	B	KwaZulu Natal	3	1	4
	H	Gauteng	5	0	5
	I	North West	11	2	13
	J	Limpopo	6	1	7
	L	Free State	9	0	9
	M	Gauteng	6	0	6
Total Saanen			**114**	**16**	**130**
Total Toggenburg			**44**	**7**	**51**
Total British Alpine			**54**	**5**	**59**
Total Dairy Goats			**212**	**28**	**240**

Animals from both stud and commercial farmers were sampled in this study (Table 3.2). A voluntary survey form was also given to the participating breeders, which was used to determine individual herd composition and the breeding practices that the specific breeders employ, such as practicing pure-breeding versus cross-breeding, methods used to source and select replacement stock and the type of records that are kept. The major products produced by the farm, as well the method of marketing were also recorded, and gives insight to the breeding goals of a specific herd, even when the farmer does not consciously implement such goals. The questionnaire used in the survey is attached as Addendum A.

Table 3.2 Number of stud and commercial Saanen, Toggenburg and British Alpine goats included in the study

Breed	**Stud**	**Commercial**
Saanen	86	44
Toggenburg	40	11
British Alpine	37	22

5ml blood was collected from the jugular vein of mature goats using a vacuum tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The samples were kept on ice while in transit to the Animal Breeding and Genetics Laboratory of the Department of Animal and Wildlife Sciences at the University of Pretoria. The blood samples were transferred into screw-top tubes, which was duplicated for each sample, and stored at -40°C until DNA extraction.

3.3 DNA Extraction and quantification

DNA was extracted from 100µl whole blood using the Qiagen DNeasy® Blood and Tissue kit (Qiagen, Hilden, Germany) following the standard protocol prescribed by the manufacturer. Extractions were done in the Animal Breeding and Genetics laboratory at the Department of Animal and Wildlife Sciences, University of Pretoria. The remaining blood was stored at -40°C.

Quantification of DNA was performed using electrophoresis in a 1% agarose gel stained with ethidium bromide, using a mixture of 3µl DNA and 2µl blue/orange loading buffer (Promega, Madison, WI, USA), on a Hoefer HE 33 Mini Horizontal Submarine Unit © (AEC-Amersham Pty. Ltd., Johannesburg, South Africa). The ethidium bromide-stained gel with the DNA was placed on UV tray and then scanned with the Gel Doc™ EZ System (Bio-Rad Laboratories Inc, Hercules, United States of America) to visualize the DNA. DNA quality was evaluated based on the intensity and clarity of the bands seen, as well as the absence of smearing patterns, which would indicate degraded DNA. DNA samples that had very faint bands or that showed degradation were discarded. Crude estimation of the DNA concentration in each sample was

done. The estimated concentration varied between samples from 50ng/µl up to 100 ng/µl. The DNA concentration used in calculations was therefore 50ng/µl.

3.4 Choice of markers

Twenty-five microsatellites were used for this study, summarized in Table 3.3. These microsatellites consists of 16 markers from the FAO/ISAG panel recommended for diversity studies in goats (FAO, 2011) that had also been successfully used in dairy goat diversity studies (Glowatzki-Mullis *et al.*, 2008; Barrera-Saldaña *et al.*, 2010; Bruno-de-Sousa *et al.*, 2011). Three markers from the abovementioned diversity studies that are not on the recommended panel (INRA040, INRA132 and OarFCB128) were also included, based on the polymorphicity observed from these studies. Six additional microsatellites were added from an Angora goat parentage panel that had been developed in the Animal Breeding and Genetics laboratory (Friedrich, 2009; Visser *et al.*, 2011b), as this panel had also been tested in a small population of Saanen goats.

Markers from the Angora parentage panel had been found to have a high amplification success rate in the Saanen population tested in the parentage study. The microsatellites were evaluated in terms of the expected polymorphicity by examining the fragment size ranges from previous studies. Larger ranges are desirable, as a greater number of alleles can be expected within such a range, and the polymorphic information content (PIC) of a marker will be correspondingly higher. The absolute expected fragment sizes of the markers were also deciding factors, as markers had to be grouped into two genotyping sets without marker ranges overlapping.

Table 3.3 Primer sequences, genome location and expected fragment sizes of the 25 microsatellite markers used for this study

Marker[1]	**Primer sequences (5'→3')**	**Chr nr**[2]	**Range (bp)**	**References**
BM1258	F: GTATGTATTTTTCCCACCCTGC R: GAGTCAGACATGACTGAGCCTG	CHI23	104-130	Glowatzki-Mullis *et al.* (2008)
BM1329	F: TTGTTTAGGCAAGTCCAAAGTC R: AACACCGCAGCTTCATCC	CHI6	170-190	Glowatzki-Mullis *et al.* (2008)
BM1818	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	CHI23	252-264	Visser *et al.* (2011b)
BM7160	F: TGGATTTTTAAACACAGAATGTGG R: TCAGCTTCTCTTTAAATTTCTCTGG	CHI22	160-190	Visser *et al.* (2011b)
CSRD247	F: GGACTTGCCAGAACTCTGCAAT R: CACTGTGGTTTGTATTAGTCAGG	OAR14	219-245	Glowatzki-Mullis *et al.* (2008)

Marker[1]	Primer sequences (5'→3')	Chr nr[2]	Range (bp)	References
HSC	F: CTGCCAATGCAGAGACACAAGA R: GTCTGTCTCCTGTCTTGTCATC	CHI23	265-301	Visser *et al.* (2011b)
ILSTS005	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	OAR7	176-190	Glowatzki-Mullis *et al.* (2008)
ILSTS011	F: GCTTGCTACATGGAAAGTGC R: CTAAAATGCAGAGCCCTACC	CHI14	260-280	Bruno-de-Sousa *et al.* (2011)
ILSTS087	F: AGCAGACATGATGACTCAGC R: CTGCCTCTTTTCTTGAGAGC	CHI6	134-154	Iamartino *et al.* (2005)
INRA023	F: GAGTAGAGCTACAAGATAAAC R: TAACTACAGGGTGTTAGATGAACTCA	CHI3	197-223	Barrera-Saldaña *et al.* (2010)
INRA040	F: TCAGTCTGGAGGAGAGAAAAC R: CTCTGCCCTGGGGATGATTG	CHI2	220-252	Glowatzki-Mullis *et al.* (2008)
INRA063	F: ATTTGCACAAGCTAAATCTAACC R: AAACCACAGAAATGCTTGGAAG	CHI18	165-199	Glowatzki-Mullis *et al.* (2008)
INRA132	F: AACATTTCAGCTGATGGTGGC R: TTCTGTTTTGAGTGGTAAGCTG	OAR20	138-146	Glowatzki-Mullis *et al.* (2008)
INRABERN172	F: CCACTTCCCTGTATCCTCCT R: GGTGCTCCCATTGTGTAGAC	CHI26	232-252	Glowatzki-Mullis *et al.* (2008)
INRABERN185	F: CAATCTTGCTCCCACTATGC R: CTCCTAAAACACTCCCACACTA	BTA18	262-290	Glowatzki-Mullis *et al.* (2008)
INRABERN192	F: AGACCTTTACAGCCACCTCTTC R: GTCCCAGAAACTGACCATTTTA	CHI7	178-198	Visser *et al.* (2011b)
MAF65	F: AAAGGCCAGAGTATGCAATTAGGAG R: CCACTCCTCCTGAGAATATAACATG	OAR15	118-160	Bruno-de-Sousa *et al.* (2011)
MAF209	F: TCATGCACTTAAGTATGTAGGATGCTG R: GATCACAAAAAGTTGGATACAACCGTGG	OAR17	104-108	Glowatzki-Mullis *et al.* (2008)

Marker[1]	Primer sequences (5'→3')	Chr nr[2]	Range (bp)	References
MCM527	F: GTCCATTGCCTCAAATCAATTC R: AAACCACTTGACTACTCCCCAA	CHI7	157-177	Visser *et al.* (2011a)
OarFCB20	F: AAATGTGTTTAAGATTCCATACAGTG R: GGAAAACCCCCATATATACCTATAC	OAR2	92-126	Iamartino *et al.* (2005)
OarFCB48	F: GACTCTAGAGGATCGCAAAGAACCAG R: GAGTTAGTACAAGGATGACAAGAGGCAC	CHI17	151-175	Bruno-de-Sousa *et al.* (2011)
OarFCB128	F: ATTAAGCATCTTCTCTTTATTTCCTCGC R: CAGCTGAGCAACTAAGACATACATGCG	OAR2	96-104	Glowatzki-Mullis *et al.* (2008)
SRCRSP5	F: GGACTCTACCAACTGAGCTACAAG R: TGAAATGAAGCTAAAGCAATGC	CHI21	161-181	Jiménez-Gamero *et al.* (2006)
SRCRSP8	F: TGCGGTCTGGTTCTGATTTCAC R: GTTTCTTCCTGCATGAGAAAGTCGATGCTTAG	CHI6	209-245	Visser *et al.* (2011b)
SRCRSP9	F: AGAGGATCTGGAAATGGAATC R: GCACTCTTTTCAGCCCTAATG	CHI12	113-143	Visser *et al.* (2011b)

[1] Markers in **bold** are from the FAO/ISAG recommended panel for goats; underlined markers are from the parentage study by Visser *et al.* (2011b)

[2] Chromosome number from Glowatzki-Mullis *et al.* (2008) and Visser (2010)

3.5 PCR amplification

PCR amplification of the DNA samples was done using 25 microsatellites, divided into two genotyping sets (12 markers and 13 markers respectively) based on the expected fragment sizes. The forward primers of the microsatellite markers were then labelled with a fluorescent dye in one the following colours: blue (6-FAM®), red (PET®), green (VIC®) or yellow (NED®). The annealing temperature (T_A) for each marker was found through conducting optimization runs. During the optimization process it was found that some markers produced a great amount of non-specific amplification, even at the "optimal" temperature. It was found that by increasing the final extension step from 5 minutes to either 20 or 45 minutes that the non-specific amplification was reduced, and the result was that the allelic profile was cleaner and easier to score. The genotyping sets are presented in Table 3.4, along with the annealing temperatures and final extension step length.

The PCR Mastermix for each sample consisted of 0.3µl each of the forward and reverse primers, which had been diluted to a concentration of 10pmol/µl beforehand using nuclease-free water, and 0.3µl Bioline MyTaq DNA polymerase (Bioline - Celtic Molecular Diagnostics (Pty) Ltd., South Africa). These components were added separately to 3µl Bioline MyTaq Buffer and 6.1µl deionised water for a volume of

10µl. 5µl of 50 ng/µl DNA was then added to make a final PCR mix volume of 15µl per sample. PCR amplification was carried out using a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, USA). The PCR consisted of: 10 minutes at 94°C, followed by 33 cycles of 45 seconds at 94°C, 80 seconds at the annealing temperature and 60 seconds at 72°C; concluded with a final extension step at 72°C. This extension step was five minutes for most of the markers, but was extended to no more than 45 minutes for markers that showed a large amount of non-specific amplification during optimization. The final extension step times can be seen in Table 3.4. Amplicons were run on a 3% agarose gel, using 3µl PCR product together with 2µl blue/orange loading buffer (Promega, Madison, WI, USA) to test for PCR success.

Table 3.4 Microsatellites grouped according to the two genotyping sets, showing the fluorescent dyes, the annealing temperatures (T_A) and the final extension step length

Primer set	Size group	Marker name	Fluorescent Dye	T_A (°C)	Final extension step
1	1 (50-150)	ILSTS087	PET®	55	5 min
	1 (50-150)	SRCRSP9	VIC®	55	5 min
	1 (50-150)	MAF65	6-FAM®	62	5 min
	1 (50-150)	MAF209	NED®	55	5 min
	2 (150-250)	BM1329	PET®	55	5 min
	2 (150-250)	BM7160	VIC®	55	5 min
	2 (150-250)	INRABERN192	6-FAM®	55	5 min
	2 (150-250)	SRCRSP5	NED®	55	5 min
	3 (250-350)	BM1818	PET®	55	20 min
	3 (250-350)	SRCRSP8	VIC®	55	5 min
	3 (250-350)	HSC	6-FAM®	62	5 min
	3 (250-350)	CSRD247	NED®	55	45 min
2	1 (50-150)	OARFCB128	PET®	48	5 min
	1 (50-150)	BM1258	VIC®	60	5 min
	1 (50-150)	OARFCB20	6-FAM®	46	20 min
	1 (50-150)	INRA132	NED®	55	45 min
	2 (150-250)	OARFCB48	PET®	60	5 min
	2 (150-250)	MCM527	VIC®	55	45 min
	2 (150-250)	INRA63	6-FAM®	55	5 min
	2 (150-250)	ILSTS005	NED®	60	5 min
	3 (250-350)	INRA23	PET®	46	20 min
	3 (250-350)	INRABERN185	PET®	55	5 min
	3 (250-350)	INRABERN172	VIC®	58	5 min
	3 (250-350)	INRA40	6-FAM®	58	5 min
	3 (250-350)	ILSTS011	NED®	60	45 min

Successful amplicons were genotyped using an ABI PRISM® 3500XL DNA Genetic Analyser (Applied Biosystems, Foster City, USA) at the FABI (Forestry and Agricultural Biotechnology Institute) sequencing laboratory of the University of Pretoria. Dilution for genotyping consisted of a one-in-ten dilution of PCR product in a Formamide:LIZ standard (in a ratio of 1000:14), with 1µl of diluted PCR product being added to 9µl Formamide-LIZ mixture.

3.6 Allele calling and statistical analysis

The alleles of the genotyped samples were called using the GeneMarker™ software (www.softgenetics.com/GeneMarker.html), thereby determining the fragment sizes. Data control was done using the Excel Microsatellite Toolkit (Park, 2001) and the polymorphic information content (PIC) was calculated. Data conversion for use in other programmes were performed with the CONVERT 1.31 (Glaubitz, 2004) software. CONVERT was also used to calculate allelic frequencies and to identify private alleles within the populations.

Analysis of the population structure was performed with the software STRUCTURE 2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003) to determine the true number of populations (K), by using Bayesian-based assignment principles. The software ignores available population information, therefore identifying distinct genetic populations, and assigns individuals based upon their genetic membership. The model used for the simulation assumes admixture in the ancestry, and therefore assumes correlated allele frequencies. The model assumed the probability of the number of populations (Ln Pr (X|K)) to be $2 \leq K \leq 9$. Five independent runs were performed for each K, and the probability value for each K was averaged over the runs. The runs were carried out with a burn-in period of 100,000 steps, followed by 500,000 Markov chain Monte Carlo (MCMC) iterations.

The assumption of admixture in the model allows for the calculation of the proportion of admixture in the ancestry of a specific individual (Pritchard *et al.*, 2000). *Q* is used to show the proportions of an animal's genome that originated from a different population, and the result of *Q* is given in a matrix. Clustering of individuals into their assigned populations is then done by grouping animals that share the greater proportion of their genome together according to the *Q*-matrix. This clustering can be graphically represented by plotting *Q* in a bar graph, giving a *Q*-plot to compare the clustering results.

FSTAT version 2.9.3.2 (Goudet, 2001) was used to calculate Wright's *F*-statistics for each locus, both over the whole population, and for each breed separately. *F* denotes Wright's F_{IT}, which calculates the inbreeding coefficient of an individual (I) relative to the total population (T). F_{IT} therefore determines heterozygote deficiency globally in a population. F_{IS} is denoted by *f*, and calculates the inbreeding coefficient of I relative to the subpopulation (S), which allows for the comparison with the inbreeding coefficient of the total population (Goudet, 2001), and therefore determines heterozygote deficiencies within the subpopulations. F_{ST} compares the heterozygote deficiencies among the populations. This is used as it may occur that two sub-populations are in Hardy Weinberg Equilibrium (HWE), but that their allele frequencies

differ, leading to a decrease in heterozygosity in the total population. This phenomenon is known as the Wahlund effect, and F_{ST} is therefore the measure used to determine this (Goudet, 2001). Wright's *F*-statistics were calculated using the method by Weir and Cockerham of 1984 (Weir & Cockerham, 1984; Goudet, 2001), which are presented in equation 3.1, equation 3.2 and equation 3.3.

Equation 3.1 Calculation of Wright's F_{IT} according to Weir & Cockerham (1984)

$$F_{IT} = \frac{\sigma_a^2 + \sigma_b^2}{\sigma_a^2 + \sigma_b^2 + \sigma_w^2}$$

Equation 3.2 Calculation of Wright's F_{ST} according to Weir & Cockerham (1984)

$$F_{ST} = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_b^2 + \sigma_w^2}$$

Equation 3.3 Calculation of Wright's F_{IS} according to Weir & Cockerham (1984)

$$F_{IS} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2}$$

Where σ_a^2 = variance among populations

σ_b^2 = variance among individuals within a population

σ_w^2 = variance among individuals

The relationship between F_{IT}, F_{ST} and F_{IS} is given by $F_{IS} = \frac{F_{IT} - F_{ST}}{1 - F_{ST}}$ (Weir & Cockerham, 1984). F_{IT}, F_{ST} and F_{IS} estimations were obtained for each locus among the populations, and significance levels were derived using a jack-knifing procedure across all loci. Nei's estimation of heterozygosity (Nei, 1987) was also obtained with FSTAT. The equation used to calculate the observed heterozygosities (H_O) can be seen in equation 3.4.

Equation 3.4 Nei's estimation of observed heterozygosity (H_O)

$$H_O = 1 - \sum_k \sum_i \frac{p_{kii}}{n_p}$$

Where n_p = number of samples

p_{kii} = frequency of genotype A_iA_i in sample *k*

The estimation of Nei's expected heterozygosities within the samples (H_S) uses the result from equation 3.4 in equation 3.5, as well as the harmonic mean (ñ) of the samples that are calculated as in equation 3.6, and the allelic frequency ($\bar{p}_i^2$) as seen in equation 3.7. The calculation of the overall heterozygosity (H_T) is presented in equation 3.8. Each of these measures of heterozygosity are independent of sample sizes.

Equation 3.5 Nei's estimation of expected heterozygosity (H_S) within samples

$$H_S = \frac{\tilde{n}}{\tilde{n}-1}\left[1 - \sum_i \bar{p}_i^2 - \frac{H_O}{2\tilde{n}}\right]$$

Where ñ = harmonic mean of sample *k*

Equation 3.6 Calculation of the harmonic mean (ñ) of sample *k*

$$\tilde{n} = \frac{n_p}{\sum_k \frac{1}{n_k}}$$

Equation 3.7 Calculation of the allelic frequency

$$\bar{p}_i^{\,2} = \sum_k \frac{p_{ki}^2}{n_p}$$

Equation 3.8 Nei's estimation of overall expected heterozygosity (H_T)

$$H_T = \frac{1 - \sum_i \bar{p}_i^{\,2} + H_S}{\left(\tilde{n}\, n_p\right) - \frac{H_O}{(2\tilde{n}\, n_p)}}$$

Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) allowed for the determination of the observed (H_O) and the expected (H_E) heterozygosities within and among the populations, and to confirm the results obtained with Nei's estimation of heterozygosity. The deviation from Hardy Weinberg equilibrium (HWE) was also determined with Arlequin, and population subdivision estimates were obtained by the calculation of the fixation index, F_{ST}. The analysis of molecular variance (AMOVA) was conducted to determine the differentiation within and between the populations.

3.7 Pedigree analysis

Pedigree records dating back to 1955 for the Saanen (4023 animals), 1960 for the Toggenburg (579 animals) and 1970 for the British Alpine (597 animals) were obtained from SA Stud Book (PO Box 270,

Bloemfontein, 9300). Particulars recorded include individual animal registration number, on-farm identification number and name, date of birth, gender, sire and dam registration numbers, as well as whether the animal is alive or not at the time that the records are requested. The herdbooks of the Saanen, the Toggenburg and the British Alpine are all managed as open herdbooks, and as such any animal that have been judged and approved for registration by the SAMGBS can be added to the appropriate herdbook. These new registrations often don't have complete pedigree records. Stud animals are more likely to be registered, while strictly commercial goats are rarely registered.

The software POPREP (Groeneveld *et al.*, 2010) was used to analyse the population structure of the breeds with the available records. The software analyses the data in terms of cohorts, where animals are grouped according to year of birth, and the animals may or may not have been selected as replacement stock. This allows for the determination of the number of breeding males and females for any given year, as well as the age structure of the parents. The age structure of the parents is also useful in determining the generation interval, and the distribution of parity is an indication of the length of time that an animal remains in the herd before being culled.

POPREP also analyses the pedigree quality by taking into consideration the completeness of the pedigrees, and producing a Pedigree Completeness Index (PCI) (Groeneveld *et al.*, 2010). The PCI is a summary of the known ancestors in each of the previous generations, and is calculated using equation 3.9. The PCI of an individual (*I*) up to a specified generation (*d*) is scored as either a 1 or 0; if either the dam or the sire is unknown a 0 is assigned, while a 1 is assigned when all the ancestors of the individual are accounted for. POPREP assumes that animals with unknown parents are unrelated, and the PCI value decreases as a pedigree's completeness decreases; therefore the probability of detecting inbreeding in a population decreases with a low PCI value, as the records are too incomplete.

Equation 3.9 Calculation of the pedigree completeness index (PCI)

$$I_d = \frac{4 I_{d_{pat}} I_{d_{mat}}}{I_{d_{pat}} + I_{d_{mat}}}$$

$$I_{d_k} = \frac{1}{d} \sum_{i=1}^{d} a_i$$

Where k = *pat* (paternal) or *mat* (maternal) line

a_i = proportion of known ancestors in generation i

d = number of generations considered

The POPREP software is also used to calculate the inbreeding coefficient F, and the rate of inbreeding change per year (ΔF). It should however be noted that when animals with unknown parents are added to the herdbook, the model assumes that these animals are unrelated to the recorded population, and consequently

assigns an inbreeding coefficient of zero to those animals. The rate of change is calculated based on the inbreeding coefficient of a cohort born in a given year, and either using the *F* of all the parents of the given cohort in comparison, or by considering the *F* of another cohort born a generation earlier, as calculated using the generation interval (Caballero, 1994; Groeneveld *et al.*, 2010). This is done for each year. The formula used in POPREP to calculate Δ*F* is presented in equation 3.10. The additive genetic relationship (AGR) is calculated in a similar fashion, and is also used in determining inbreeding levels in a population.

Equation 3.10 Formula used by POPREP to calculate inbreeding rate of change (Δ*F*)

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$$

Where F_t = inbreeding coefficient of given cohort

F_{t-1} = inbreeding coefficient of cohort used for comparison

The effective population size (N_e) is determined in POPREP by several different methods. It firstly uses a defined number of generations, and an equal number of breeding males and females (equation 3.11). This method will be designated as Method 1 throughout this dissertation. This method tends to overestimate the N_e, and is used when other methods cannot be used due to lack of data (Falconer, 1989; Groeneveld *et al.*, 2010). The overestimation of N_e occurs because the number of males in a traditional breeding population is normally far outnumbered by the number of breeding females, and a 1:1 ratio is therefore rarely seen. As Method 1 relies on an equal number of males and females being present, the influence of the sex that is in the minority – in this case the breeding males – has a greater influence on the eventual estimate of the N_e (Falconer, 1989).

Equation 3.11 Formula used by POPREP to calculate effective population size (N_e) by considering the number of breeding males and females within a discrete generation interval

$$N_e = \frac{4N_m N_f}{N_m + N_f}$$

Where N_m = number of breeding males

N_f = number of breeding females

A more accurate method makes use of the inbreeding rate of change (Δ*F*), as can be seen in equation 3.12. N_e will show a variation depending on whether the entire population (breeding and non-breeding animals) were used to calculate Δ*F* in equation 3.10, or only the actual parents of the successive generations. For this study, only the breeding population was used to determine Δ*F*. This method will be designated as Method 2 throughout this dissertation. Method 2 is dependent on the degree of pedigree completeness; the

greater the PCI of a population, the better the chance of detecting inbreeding in the population. When individuals with unknown parentage are added to the herdbook however, the PCI as well as the average inbreeding coefficient of the population decreases. This will then cause the ΔF to either decrease or remain constant. N_e was not computed in the years where $\Delta F \leq 0$, as it is undefined.

Equation 3.12 Formula used by POPREP to calculate effective population size (N_e) by considering the inbreeding rate of change (ΔF) of the parents

$$N_e = \frac{1}{2\Delta F}$$

Both Method 1 and Method 2 were used in this study to determine the effective population sizes of the Saanen, Toggenburg and British Alpine breeds, using the records that were available in their respective herdbooks. It should be borne in mind that only a fraction of the dairy goat population in South Africa is recorded in the herdbook, and as such these two methods only provide a range in which the true N_e of these breeds may occur.

Chapter 4

Results

4.1 Introduction

The sampled populations of the Saanen (130), Toggenburg (51) and British Alpine (59) breeds were genotyped with 25 microsatellite markers. All microsatellites amplified successfully, and an average amplification success rate of 99.5% was achieved. The lowest rate of amplification was seen in INRA40, where only 235 of the 240 goats (97.9%) could be assigned a genotype. The amplification success rate for each individual marker is attached as Addendum B. None of the microsatellites were discarded due to amplification failure and none were found to be monomorphic. All 25 microsatellites were therefore included in the statistical analysis and the determination of the population structure.

A voluntary survey form was given to the breeders from which animals were sampled. This was used to determine individual herd composition, product focus and the breeding practices that these breeders employ, the results of which are reported here. An analysis of the recorded pedigrees of the Saanen, Toggenburg and British Alpine studbooks was performed using POPREPORT (Groeneveld *et al.*, 2010). This was used to calculate the effective population size and inbreeding levels in each of these populations.

4.2 Survey results

A voluntary survey form was given to each of the thirteen breeders where animals were sampled. Nine responses were received. Three of these breeders have been in existence for more than ten years, with the oldest stud breeding dairy goats for the past 36 years. Four of the nine breeders started breeding goats in the last five years, and the youngest stud included in this study is two years old.

The herd composition of the breeders can be seen in Figure 4.1. Eight of the nine breeders indicated the number of animals they had, while one only indicated the breeds that they kept. Four of the breeders only kept a single breed, of which the most popular was the Saanen. Of the remaining five breeders, two routinely practice crossbreeding, which is believed to increase the milk solids and volumes through hybrid vigour. One of the breeders that keeps all three breeds and practices pure-line breeding has indicated that the stud will be dispersing their British Alpine and Toggenburg goats in the near future in order to concentrate only on their Saanen herd. Another breeder that kept only Saanen indicated that the stud will be acquiring Toggenburg goats for crossbreeding purposes to increase their milk solids. One breeder had 450 Saanen does in milk, while another had 400. The smallest herd was 47 animals in total; consisting of 18 British Alpine and 12 Toggenburg does in milk.

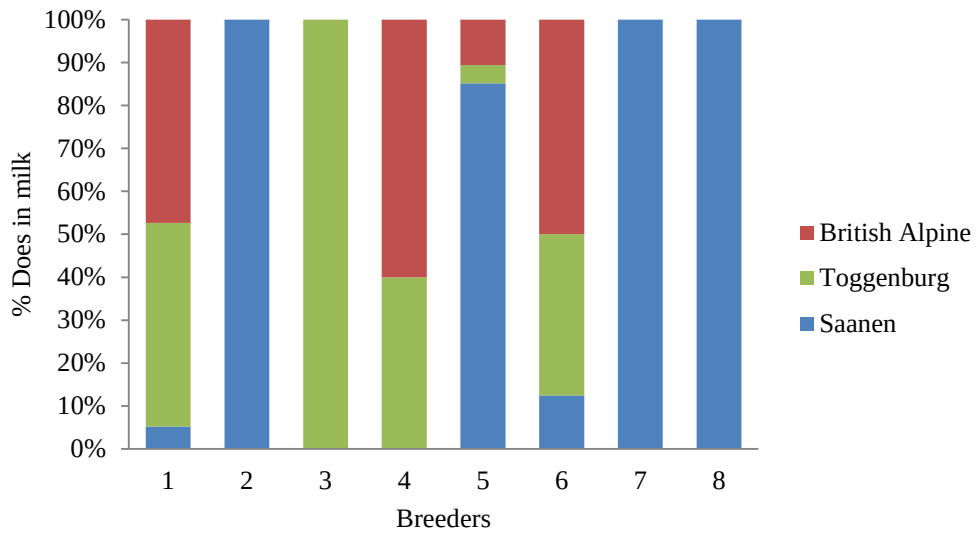


Figure 4.1 A proportionate comparison of the herd composition of breeders participating in the survey

Three breeders indicated that they make use of laprascopic artificial insemination as well as natural service. Only one breeder imports replacement stock, while the remaining breeders either source their replacement does and bucks from local co-breeders, or breeds their own. Four of the breeders indicated that they use some form of selection criteria in selecting replacement stock, which most often consists of a selection index, including mainly traits such as milk production and butterfat content. All of the breeders would make excess stock available for sale. Record-keeping practices among the breeders were variable. Two of the breeders kept no records, while one other only kept pedigree records. Four of the breeders that kept both pedigree and production records indicated that they also participate in the national recording scheme.

The products produced by these breeders are indicated in Figure 4.2. The most popular products are soft cheeses, followed by fresh milk and hard cheeses. Two breeders also manufacture other products, such as kefir – a fermented milk and grain product - and goat's milk soap. Four of the breeders market their products directly to industry, while another four market directly to the public through on-farm sales or informal markets. One breeder uses both of these marketing channels.

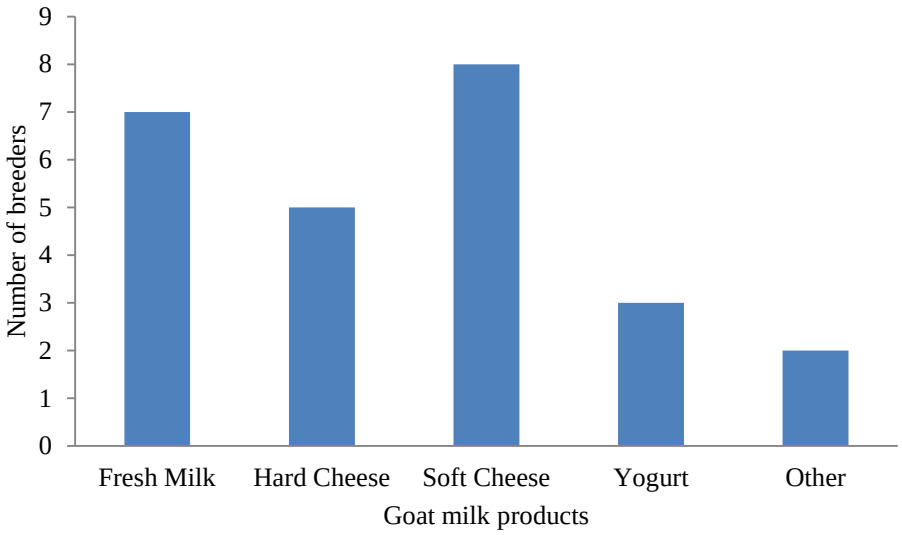


Figure 4.2 Goat milk products produced by breeders participating in the survey

4.3 Genetic characterization

Allelic frequencies

In Table 4.1 a summary of the alleles identified in the study, as well as the most and least frequent alleles found in each breed are shown. The complete table of allelic frequencies is attached as Addendum C. None of the microsatellites were monomorphic, and therefore all microsatellites were included in the statistical analysis. 201 different alleles were detected across the 25 microsatellite markers analysed from 240 genotyped individuals. The mean number of observed alleles across all populations was 8.0, with the lowest number being three alleles (MAF209) ranging up to twelve alleles (HSC, SRCRSP8, BM1258) over all populations. Within the populations, the British Alpine had the highest mean number of alleles (6.84 ± 2.08), closely followed by the Saanen (6.80 ± 2.47) and the Toggenburg (6.44 ± 2.42).

Table 4.1 Summary of alleles identified, showing the most and least frequent alleles and their (frequencies)

Locus	n	Most frequent alleles			Least frequent alleles		
		Saanen	Toggenburg	British Alpine	Saanen	Toggenburg	British Alpine
BM1258	12	103 (0.29)	103 (0.29)	101 (0.23)	123 (0.01)	111 (0.01)	99, 113 (0.02)
BM1329	8	178 (0.38)	178 (0.48)	178 (0.69)	176, 182 (0.01)	182 (0.01)	176, 180, 182 (0.01)
BM1818	7	256 (0.33)	256 (0.44)	258 (0.29)	254 (0.04)	254 (0.03)	266 (0.03)
BM7160	8	175 (0.31)	177 (0.28)	175 (0.58)	183 (0.01)	169 (0.01)	167 (0.03)
CSRD247	8	233 (0.55)	233 (0.52)	233 (0.44)	241 (0.01)	239, 245 (0.01)	235 (0.02)
HSC	12	283 (0.30)	281 (0.30)	283 (0.54)	277 (0.01)	287, 289, 291, 299 (0.01)	291 (0.01)
ILSTS005	5	181 (0.80)	181 (0.60)	181 (0.49)	177, 189 (0.02)	177 (0.03)	179 (0.01)
ILSTS011	9	277 (0.37)	279 (0.39)	277 (0.40)	283 (0.02)	281 (0.02)	281 (0.01)
ILSTS087	10	153 (0.50)	145 (0.42)	145 (0.48)	155, 157 (0.01)	157 (0.05)	147 (0.01)
INRA23	9	213 (0.74)	213 (0.58)	213 (0.72)	205 (0.01)	207 (0.01)	197 (0.01)
INRA40	11	236 (0.40)	244, 246 (0.27)	244 (0.26)	224 (0.01)	222, 248 (0.01)	224 (0.01)
INRA63	5	165 (0.50)	165 (0.4412)	167 (0.41)	169 (0.02)	161 (0.02)	169 (0.01)
INRA132	7	139 (0.49)	139 (0.60)	139 (0.47)	137 (0.01)	131, 151 (0.01)	151, 155 (0.02)
INRABERN172	8	245 (0.43)	245 (0.52)	239 (0.26)	233 (0.02)	247 (0.01)	241 (0.01)
INRABERN185	5	265 (0.67)	265 (0.73)	265 (0.79)	287 (0.13)	267 (0.02)	287 (0.01)
INRABERN192	9	182 (0.42)	186 (0.67)	186 (0.57)	188, 196 (0.01)	198 (0.01)	184 (0.01)
MAF65	9	132 (0.32)	134 (0.43)	132 (0.41)	126 (0.01)	120 (0.01)	122 (0.01)
MAF209	3	107 (0.77)	107 (0.86)	107 (0.74)	105 (0.04)	105 (0.05)	109 (0.07)
MCM527	7	155 (0.47)	155 (0.47)	155 (0.64)	167 (0.02)	169, 173 (0.01)	165 (0.04)
OarFCB20	6	95 (0.33)	95 (0.87)	95 (0.53)	91 (0.01)	101 (0.01)	91 (0.01)
OarFCB48	7	168 (0.37)	164 (0.43)	168 (0.36)	160 (0.04)	156 (0.02)	156, 160 (0.03)

Locus	n	Most frequent alleles			Least frequent alleles		
		Saanen	Toggenburg	British Alpine	Saanen	Toggenburg	British Alpine
OarFCB128	4	100 (0.66)	100 (0.94)	100 (0.74)	104 (0.01)	102 (0.06)	98 (0.02)
SRCRSP5	9	172 (0.74)	172 (0.37)	172 (0.73)	178, 182 (0.01)	164 (0.05)	180 (0.01)
SRCRSP8	12	247 (0.38)	247 (0.27)	237 (0.32)	243 (0.01)	219, 243 (0.01)	217, 239, 241, 243, 249 (0.01)
SRCRSP9	11	128 (0.31)	126 (0.60)	126 (0.41)	124 (0.01)	136 (0.01)	120 (0.01)
Average	8						

n: number of alleles

Alleles that were observed with low frequencies, as well as those seeming to be unique to certain populations, were checked for genotyping errors, and confirmed as read. The alleles unique to a population were designated as private alleles, and are shown in Table 4.2. The Toggenburg had six private alleles in six markers, the lowest number, while the Saanen had thirteen private alleles found in nine markers, and the British Alpine fourteen private alleles in eleven markers. The private allele with the highest frequency was found in the Saanen, where allele 215 of INRA23 could be found in 3.6% of the population. Of the 33 private alleles identified, only four occurred in more than 1% of the sampled populations – two each in the Saanen and British Alpine. None of the private alleles identified in the Toggenburg had a frequency greater than 0.005.

Table 4.2 Private alleles found in the Saanen, Toggenburg and British Alpine populations and their (frequencies)[1]

Locus	Saanen	Toggenburg	British Alpine
BM1258	123 (0.002)		
BM7160	183 (0.004)		
CSRD247		245 (0.002)	
HSC	277 (0.004)	299 (0.002)	
ILSTS005	189 (0.008)		179 (0.002)
ILSTS011			**273 (0.013)**; 275 (0.008)
ILSTS087	155 (0.002)		**139 (0.015)**; 147 (0.002)
INRA23	**215 (0.036)**		197 (0.002)
INRABERN172			251 (0.004)
INRABERN185		267 (0.004)	277 (0.004)
INRABERN192	176 (0.002); 196 (0.002)	198 (0.002)	184 (0.002)
MCM527		173 (0.002)	
OarFCB48			172 (0.004)
OarFCB128			98 (0.004)
SRCRSP5	176 (0.002); 182 (0.002)		180 (0.002)
SRCRSP8		219 (0.002)	241 (0.002); 249 (0.002)
SRCRSP9	**122 (0.013)**; 130 (0.002); 138 (0.002)		

[1]Alleles with a frequency >1% in **bold**

Genetic diversity

A summary of the average level of genetic diversity, population subdivision and polymorphic information content (PIC) is shown in Table 4.3. The overall genetic diversity was moderate in all three breeds, varying from 62.6% to 63.4%. In the Saanen and the British Alpine breeds, the average unbiased expected heterozygosity (H_E) was higher than the average observed heterozygosity (H_O), while in the Toggenburg the average H_O was higher than the average H_E. The F_{ST} value was very similar for the Saanen (0.050), Toggenburg (0.053) and British Alpine (0.052). The values obtained for each locus per breed are attached as Addendum D. The PIC values of the 25 markers in the Saanen breed were low to moderate, with values ranging from 0.30 to 0.81, averaging at 0.60. Although the range of the PIC values in the Toggenburg and the British Alpine were greater, the average PIC values were similar to that of the Saanen.

Table 4.3 Summary statistics estimated for the Saanen, Toggenburg and British Alpine populations genotyped with 25 microsatellites

Population	**Sample size**	**Loci typed**	**Unbiased Hz ± SD**	**Obs Hz ± SD**	**n Alleles ± SD**	F_{ST}	**PIC**
Saanen	130	25	0.650 ± 0.0300	0.626 ± 0.0085	6.80 ± 2.47	0.050	0.603
Toggenburg	51	25	0.624 ± 0.0388	0.634 ± 0.0135	6.44 ± 2.42	0.053	0.577
British Alpine	59	25	0.641 ± 0.0291	0.634 ± 0.0126	6.84 ± 2.08	0.052	0.596

The 25 microsatellite markers were also tested for deviation from Hardy Weinberg Equilibrium (HWE) (Table 4.4) within each population. In the Saanen, it was found that 20 of the 25 loci were in Hardy Weinberg equilibrium ($P > 0.05$). The British Alpine and the Toggenburg each had six loci that deviated significantly from HWE. One locus (INRA40) had a deviation from HWE in all three breeds, while three more loci (ILSTS011, MAF209 and OarFCB128) deviated only in the Toggenburg and British Alpine, and BM1258 deviated from HWE only in the Saanen and British Alpine.

Table 4.4 Hardy Weinberg Equilibrium (HWE) deviations for each of the 25 microsatellite markers in the Saanen, Toggenburg and British Alpine populations[1]

Locus	Saanen	Toggenburg	British Alpine
BM1258	**0.00873 ± 0.00007**	0.21657 ± 0.0003	**0.00998 ± 0.00014**
BM1329	0.96955 ± 0.00019	0.99764 ± 0.00005	0.48968 ± 0.00042
BM1818	0.97654 ± 0.00015	0.78515 ± 0.00034	0.22899 ± 0.00035
BM7160	**0.00231 ± 0.00004**	0.16582 ± 0.00038	0.09948 ± 0.00031
CSRD247	0.27633 ± 0.00041	0.98022 ± 0.00012	0.16934 ± 0.00035
HSC	0.25038 ± 0.00039	0.29218 ± 0.00027	0.66082 ± 0.00034
ILSTS005	0.70515 ± 0.0005	0.44729 ± 0.00047	0.13874 ± 0.00032
ILSTS011	0.87705 ± 0.00031	**0.00066 ± 0.00002**	**0.00006 ± 0.00001**
ILSTS087	0.19619 ± 0.00038	0.21946 ± 0.00036	0.75546 ± 0.0005
INRA23	0.84912 ± 0.00026	0.27328 ± 0.00029	**0.02354 ± 0.00016**
INRA40	**0 ± 0**	**0.00006 ± 0.00001**	**0.00008 ± 0.00001**
INRA63	**0.0122 ± 0.00011**	0.6009 ± 0.0005	0.05094 ± 0.0002
INRA132	**0.02683 ± 0.0002**	0.1598 ± 0.00031	0.21888 ± 0.00033
INRABERN172	0.11217 ± 0.00029	0.13829 ± 0.00031	0.68044 ± 0.00046
INRABERN185	0.07728 ± 0.00029	**0.00837 ± 0.00008**	0.82616 ± 0.00042
INRABERN192	0.3426 ± 0.00033	0.98446 ± 0.00012	0.54706 ± 0.00051
MAF65	0.48483 ± 0.00044	**0.01518 ± 0.00012**	0.34274 ± 0.00054
MAF209	0.25925 ± 0.00044	**1 ± 0**	**1 ± 0**
MCM527	0.16641 ± 0.00035	0.57682 ± 0.00064	0.06143 ± 0.00023
OarFCB20	0.77423 ± 0.00034	0.57755 ± 0.00041	0.07491 ± 0.00029
OarFCB48	0.79465 ± 0.00036	0.57206 ± 0.00041	0.69036 ± 0.00048
OarFCB128	0.66586 ± 0.00046	**1 ± 0**	**0.03279 ± 0.00018**
SRCRSP5	0.26348 ± 0.00033	0.61175 ± 0.0004	0.27939 ± 0.00038
SRCRSP8	0.67661 ± 0.00044	0.22319 ± 0.00030	0.45101 ± 0.00033
SRCRSP9	0.11818 ± 0.00022	0.56478 ± 0.00047	0.73862 ± 0.00036

[1]Hardy Weinberg Equilibrium deviations in **bold**

Evaluation of population differentiation was done for each of the breeds, using the fixation indices (F_{IS}, F_{IT} and F_{ST}), which are presented in Table 4.5. In the Saanen, the mean estimates of the *F*-statistics found by jackknifing were F_{IT} = 0.046 ± 0.017, F_{ST} = 0.05 ± 0.005 and F_{IS} = -0.005 ± 0.017. These estimates were F_{IT} = -0.006 ± 0.020, F_{ST} = 0.053 ± 0.008 and F_{IS} = -0.063 ± 0.020 in the Toggenburg and F_{IT} = 0.019 ± 0.017, F_{ST} = 0.052 ± 0.008 and F_{IS} = -0.035 ± 0.015 in the British Alpine.

From the F_{IS}-values, it was found that three markers (INRA40, BM1258 and INRABERN172) had a significant deficiency of heterozygotes in the Saanen breed, found on CHI2, CHI23 and CHI26 respectively. Only INRA23 (CHI3) had a significant heterozygote deficiency in the British Alpine, while no significant

heterozygote deficiency in any of the markers was seen in the Toggenburg. None of the markers that had a heterozygote deficiency in any of the breeds were found on the same chromosome.

Table 4.5 Wright's *F*-statistics for the Saanen, Toggenburg and British Alpine populations, for each of the 25 microsatellite markers[1]

Locus	**Saanen**			**Toggenburg**			**British Alpine**		
	F_{IT} *(F)*	F_{ST} (Θ)	F_{IS} *(f)*	F_{IT} *(F)*	F_{ST} (Θ)	F_{IS} *(f)*	F_{IT} *(F)*	F_{ST} (Θ)	F_{IS} *(f)*
BM1258	0.160	0.065	**0.101**	0.057	0.081	-0.027	0.113	0.088	0.027
BM1329	-0.006	0.018	-0.024	-0.015	0.118	-0.152	-0.040	0.136	-0.204
BM1818	0.001	0.085	-0.093	-0.055	0.079	-0.146	-0.086	0.021	-0.110
BM7160	0.101	0.058	0.045	0.054	-0.020	0.073	0.108	0.064	0.047
CSRD247	0.010	0.018	-0.008	-0.042	0.040	-0.085	-0.070	0.031	-0.105
HSC	0.044	0.036	0.009	0.005	0.008	-0.002	0.004	-0.007	0.011
ILSTS005	-0.019	0.049	-0.071	0.036	0.009	0.028	-0.073	0.012	-0.086
ILSTS011	-0.037	0.030	-0.069	-0.251	0.002	-0.254	0.041	0.014	0.027
ILSTS087	0.023	0.071	-0.051	-0.001	0.053	-0.057	-0.116	0.022	-0.141
INRA23	0.036	0.055	-0.019	0.010	0.010	0.000	0.286	0.081	**0.223**
INRA40	0.224	0.052	**0.182**	0.018	0.061	-0.046	0.002	0.060	-0.062
INRA63	0.094	0.085	0.010	0.075	0.074	0.002	0.139	0.111	0.031
INRA132	0.083	0.001	0.082	0.202	0.123	0.090	0.014	0.040	-0.027
INRABERN172	0.131	0.035	**0.100**	-0.035	0.052	-0.092	0.090	0.042	0.050
INRABERN185	-0.051	0.050	-0.106	-0.036	0.060	-0.102	-0.103	0.064	-0.179
INRABERN192	0.088	0.071	0.017	-0.061	0.012	-0.075	0.035	0.034	0.001
MAF65	-0.018	0.068	-0.092	-0.029	0.078	-0.116	0.013	0.043	-0.032
MAF209	0.046	0.050	-0.005	-0.089	0.101	-0.212	-0.049	0.045	-0.098
MCM527	-0.101	0.061	-0.172	0.040	0.044	-0.004	0.160	0.140	0.023
OarFCB20	-0.028	0.012	-0.040	-0.002	0.027	-0.030	-0.056	-0.005	-0.051
OarFCB48	0.007	0.063	-0.060	-0.179	0.038	-0.225	-0.018	0.037	-0.058
OarFCB128	0.050	0.028	0.022	-0.050	0.014	-0.065	-0.013	-0.012	-0.001
SRCRSP5	0.032	0.013	0.019	-0.067	0.074	-0.152	0.078	0.146	-0.079
SRCRSP8	0.016	0.055	-0.041	0.142	0.088	0.060	0.021	0.069	-0.051
SRCRSP9	0.162	0.094	0.075	0.022	0.070	-0.052	-0.007	0.065	-0.077
Average ± SD	0.046 ± 0.017	0.05 ± 0.005	-0.005 ± 0.017	-0.006 ± 0.020	0.053 ± 0.008	-0.063 ± 0.020	0.019 ± 0.017	0.052 ± 0.008	-0.035 ± 0.015

[1]Heterozygote deficiency indicated in **bold**

More than half of the markers (56%) showed a negative F_{IS} value, while the remaining eleven had low positive values in the Saanen breed. In the Toggenburg nineteen of the 25 markers (76%) had negative F_{IS}

values, while the remaining six had low positive values. The British Alpine had fewer markers with a negative F_{IS} value than the Toggenburg, but more than the Saanen (64%). Nine of the markers had a low positive F_{IS} value in the British Alpine. The average F_{IS} values for the Toggenburg (-0.063) and the British Alpine (-0.035) were also low negative values, similar to that seen in the Saanen (-0.005). These low negative F_{IS} values indicate very limited inbreeding in the respective breeds. The F_{ST} value of the Saanen was found to be 0.05. The F_{ST} value in the Toggenburg and British Alpine breeds were also low positive, and very similar to that seen in the Saanen (0.053 and 0.052 respectively).

Wright's *F*-statistics were obtained for all three breeds over all the loci (Addendum E), in order to determine the relationship between the breeds. The only markers that still showed a heterozygote deficiency was BM1258 and INRA40 in the Saanen. Twelve of the 25 markers had negative F_{IS} values, while thirteen had low positive values. The mean estimates of the *F*-statistics found by jackknifing over all the populations were $F_{IT} = 0.083 \pm 0.013$, $F_{ST} = 0.064 \pm 0.007$ and $F_{IS} = 0.020 \pm 0.013$. The F_{ST} value (0.064) is similar to the values found for the Saanen, Toggenburg and British Alpine in the previous section (0.050, 0.053 and 0.52 respectively).

In order to explain the partitioning of the genetic variation seen in the Saanen, Toggenburg and British Alpine breeds, an analysis of molecular variance (AMOVA) was done, which is shown in Table 4.6. This revealed similar results to the F_{ST} estimate, showing that most of the variation seen is due to the differences in the individuals themselves (91.7%), while 6.4% of the variation is due to differentiation between breeds. A small amount of differentiation (1.9%) is due to the breed effect within populations, which confirms the results obtained with Wright's *F*-statistics.

Table 4.6 AMOVA analysis for the Saanen, Toggenburg and British Alpine populations

Source of variation	**Sum of squares**	**Variance components**	**Percentage variation**	***P*-value**
Among populations	174.331	0.54960	6.40455	0.001
Among individuals within populations	1931.945	0.16097	1.87586	0.001
Within individuals	1880.000	7.87079	91.71959	0.001
Total	3986..276	8.58136		

4.4 Population structure analysis

The population structure and level of admixture was measured using the software STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003). In Figure 4.3 the estimated probabilities (Ln Pr) of the number of true populations (K) are given. Ln Pr (X|K) increased distinctly from K = 2 to K = 6, after which it dropped suddenly at K = 7. The variation seen in K = 7 to K = 9 also increased in comparison to K = 2 to K = 6, and therefore K = 6 was assumed to be the most probable inferred number of populations. This result was in contrast to the expectation when analysing three populations.

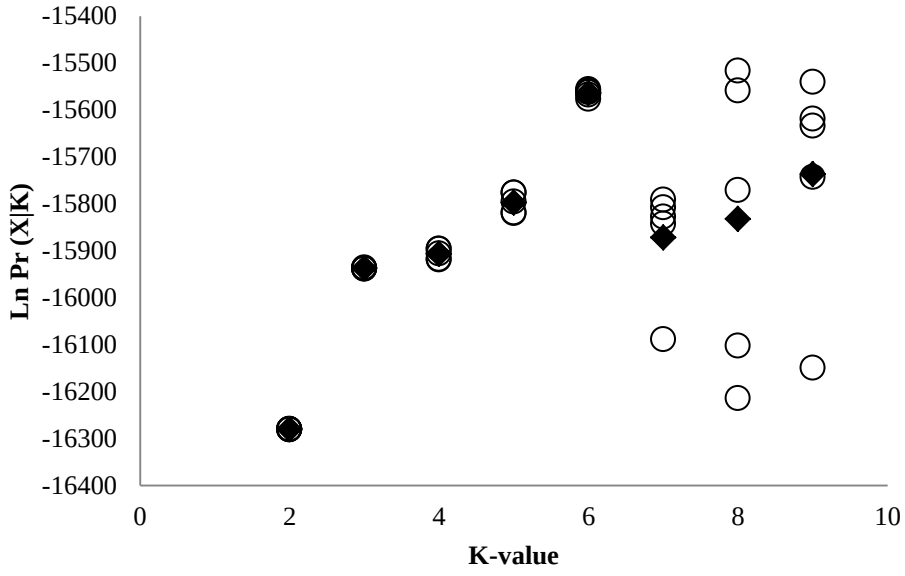


Figure 4.3 Plot of estimated probabilities of the data [Ln Pr (X|K)] for different numbers of inferred clusters (K = 2 to 9), with representation of probabilities obtained for individual runs (○) and for the mean of five runs (♦) at each K

An analysis was performed assuming three populations (K = 3), it can be seen from Table 4.7 that despite the inferred number of populations being K = 6, the three breeds do cluster together as expected. The Saanen population was mainly assigned to cluster 1 (88.4%), while 83.9% of the Toggenburg were assigned to cluster 2, and 77.9% of the British Alpine were assigned to cluster 3. The proportion of membership (*Q*) of each individual to the three clusters can be seen in the bar plot shown in Figure 4.4. Each individual is represented by a single vertical line, broken into K coloured segments, with lengths proportional to each of the three inferred clusters.

Table 4.7 Proportion of membership of each pre-defined population in each of the three clusters inferred by the STRUCTURE software[1]

Predefined populations	**Inferred clusters**			**n**
	1	**2**	**3**	
Saanen	**0.884**	0.059	0.057	130
Toggenburg	0.066	**0.839**	0.095	51
British Alpine	0.113	0.108	**0.779**	59

n: number of individuals

[1] Major clusters in **bold**

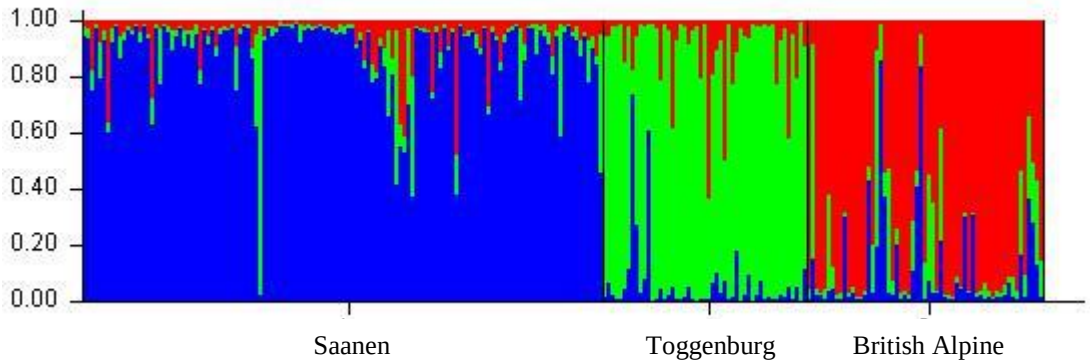


Figure 4.4 A summary plot of the inferred populations using the *Q*-matrix at K = 3

The inferred number of populations was however at K = 6, and when investigating the distribution of individuals seen in Table 4.8, it can be seen that the Saanen breed clusters into 3 distinct groups, namely clusters 4 (21.6%), 5 (35.6%) and 6 (31.0%). The proportion of Saanen clustering together has decreased only slightly from 88.4% to 88.2%. The Toggenburg still forms a single cluster, although its membership has dropped to 72.8%, and most of the British Alpine can be seen in cluster 1 (59.6%). Cluster 3 has individuals with membership from all three breeds, as can be seen from Figure 4.5, where cluster 3 is depicted in dark blue.

Table 4.8 Proportion of membership of each pre-defined population in each of the six clusters inferred by the STRUCTURE software[1]

Predefined populations	**Inferred clusters**						**n**
	1	**2**	**3**	**4**	**5**	**6**	
Saanen	0.022	0.026	0.067	**0.216**	**0.359**	**0.310**	130
Toggenburg	0.046	**0.728**	0.141	0.048	0.015	0.021	51
British Alpine	**0.596**	0.056	0.262	0.025	0.031	0.030	59

n: number of individuals

[1] Major clusters in **bold**

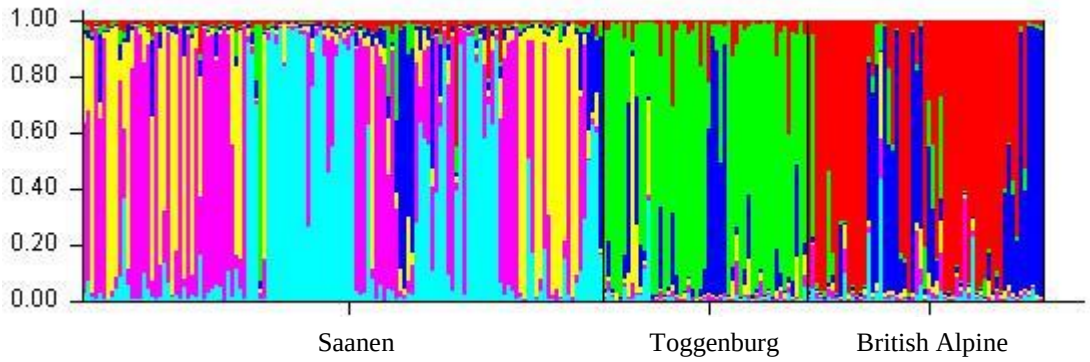


Figure 4.5 A summary plot of the inferred populations using the *Q* matrix at K = 6

When sorting the information seen in Figure 4.5 according to the *Q* values, the different clusters and the proportion of membership of each individual can be more clearly seen. From Figure 4.6 it can be seen that the Saanen have formed three closely related clusters. The Toggenburg has a single cluster, and the British Alpine another, although much smaller cluster when compared to Figure 4.4.

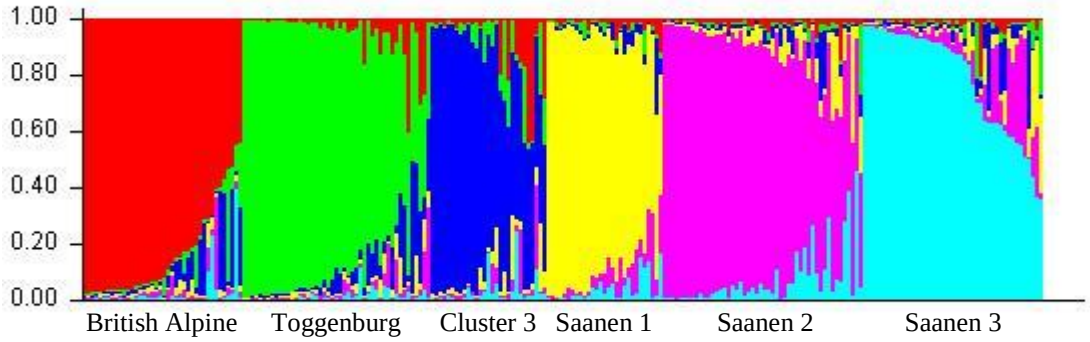


Figure 4.6 A summary plot of the 240 goats arranged according to their membership to one of the six inferred populations

It was observed that cluster 3 consists mainly of British Alpine and Toggenburg individuals, with a smaller number of Saanen individuals. Of the 29 individuals found in cluster 3, 12 originate from Farm H, while another six, five and four were sampled from Farms M, B and I respectively. At least one of the goats found in this cluster are registered in the relevant herdbook. The composition of cluster 3 is more easily observed when expanding Figure 4.6 to show the individual animals with their laboratory number and original breed membership (attached as Addendum F).

In Figure 4.7 the Saanen goats found in the three Saanen clusters are depicted along with their original sampling locations. In Saanen cluster 2 two of the main contributors to this cluster are from the Western Cape and Limpopo (Farms A and J). Saanen 1 consists of goats mainly from the Western Cape and Gauteng (Farms A and J), while Saanen 3 has goats from Limpopo, KwaZulu Natal and the Western Cape.

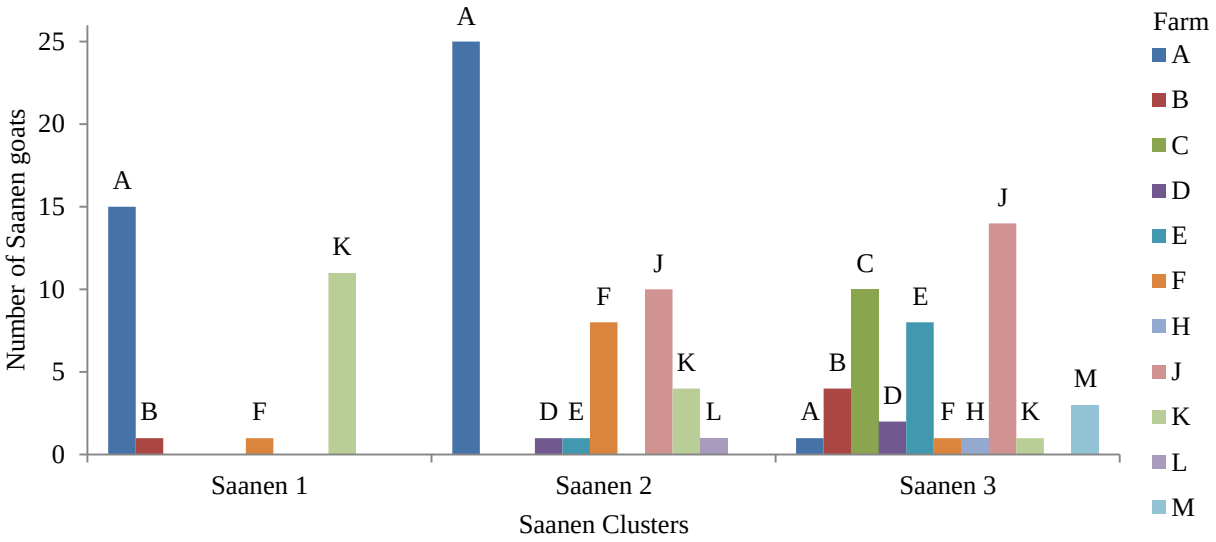


Figure 4.7 Sampling locations of the goats in Saanen clusters 1, 2 and 3

4.5 Pedigree analysis

Pedigree analysis of each of the three breeds were done using the POPREP software (Groeneveld *et al.*, 2010) from pedigree records provided by SA Stud Book. Pedigree records date back to 1955 for the Saanen (4023 animals), 1960 for the Toggenburg (579 animals) and 1970 for the British Alpine (597 animals).

In the Saanen, it was found that the average age of breeding bucks were 2.0 years, compared to 2.8 years in the Toggenburg and 2.1 years in the British Alpine (Table 4.9). A similar trend was seen in the does, where the Saanen does (1.9 years) tended to be younger on average than the British Alpine (2.1 years) and Toggenburg does (3 years). The average generation interval for selected progeny across the different populations was 3.4 years in the Saanen, 3.9 years in the Toggenburg and 3.2 years in the British Alpines. The generation interval for selected Saanen and Toggenburg does tended to be shorter than for selected bucks (3.4 and 3.7 years versus 3.5 and 4.1 years respectively). This trend is reversed in the British Alpine, where the generation interval was shorter for selected males (2.8 years) in comparison to that of the selected females (3.5 years).

Table 4.9 A comparison of the average generation intervals, breeding ages and family sizes of the Saanen, Toggenburg and British Alpine

Breed	Generation Interval			Breeding Age		Family Size	
	♂	♀	Average	♂	♀	♂	♀
Saanen	3.5	3.4	3.4	2.0	1.9	5.9	1.9
Toggenburg	4.1	3.7	3.9	2.8	3.0	3.8	1.6
British Alpine	2.8	3.5	3.2	2.1	2.1	4.6	1.8

As expected, the family sizes of sires are much larger than those of dams, with Saanen, Toggenburg and British Alpine sires averaging 5.9, 3.8 and 4.6 offspring each (Table 4.9). In comparison Saanen dams averaged 1.9 offspring, while Toggenburg dams averaged 1.6 offspring and the British Alpine dams had an average of 1.8 kids each. The largest family size for a Saanen sire to date is 67 offspring, followed by 30 offspring sired by a British Alpine and 27 kids sired by a Toggenburg buck. The largest number of offspring in the British Alpine population from a single dam is 14 kids, followed by 12 kids from one Saanen dam and 6 kids from a Toggenburg doe.

Table 4.10 Number of parities for all recorded Saanen, Toggenburg and British Alpine does up to 2012

Parity	1	2	3	4	5	6	7	8
Saanen	1746	467	129	44	14	6	3	1
Toggenburg	271	61	24	3	0	0	0	0
British Alpine	271	85	36	11	4	2	1	0

In Table 4.10 the number of parities over the recording period until 2012 for the Saanen, Toggenburg and British Alpine does is shown. It can be seen that there is a sharp decline in does that remain in the herd past their second parity, while no doe has had more than eight parities. In 2012, 188 Saanen does were in their first parity, while 27 were in their third, and one doe was in her seventh. In comparison, 27 British Alpine does that were in their first parity, while nine were in their third in the same year. There were 7 Toggenburg does were in their first parity in 2012, while four were in their third. No recorded Toggenburg doe has completed more than 4 lactations.

In Figure 4.8 the Pedigree Completeness Index (PCI) of the first generation Saanen, Toggenburg and British Alpine records are shown for the period between 1990 and 2012. The fluctuation seen in the PCI can possibly be attributed to the open herdbook system employed by the three breeds, where animals with previously unregistered parents can be added to the herdbook based on an inspection of the animal. The first generation pedigree records of the Saanen, Toggenburg and British Alpine were 100% complete in 2012. The sixth generation Saanen records were 70% complete, while the pedigree records over the 4023 animals

were 71% complete. The sixth generation records of the Toggenburg goats were 73.6% complete, with an overall completeness of 73%, while the overall completeness of the British Alpine records was 83%.

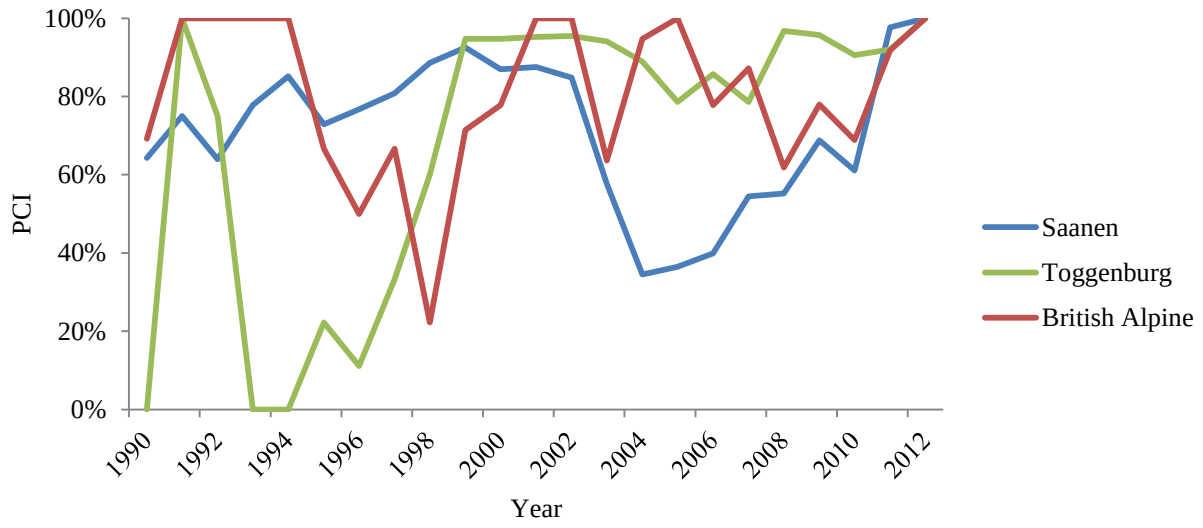


Figure 4.8 Pedigree Completeness Index (PCI) of first generation records of Saanen, Toggenburg and British Alpine kids registered between 1990 and 2012

The average inbreeding coefficients of the Saanen, Toggenburg and the British Alpine kids born between 1992 and 2012 are presented in Figure 4.9. An animal was considered inbred if its inbreeding coefficient was more than 0.05 (Groeneveld *et al.*, 2010). Of the 488 Saanen born in 2012, 347 were inbred, with an average inbreeding coefficient (F) of 0.0623 ± 0.0759 across the whole Saanen kid crop of 2012. These were the offspring of 41 inbred sires (F = 0.0642) and 152 inbred dams (F = 0.0460). The maximum F found was 0.3471. For the same year, 22 Toggenburg kids were born, of which 17 were inbred (F = 0.1335 ± 0.1063). All four of the 2012 sires were considered inbred (average F = 0.1222), while 12 of the 18 dams had an average F coefficient of 0.1058. The maximum inbreeding coefficient seen in the 2012 Toggenburg crop was 0.4043. In the British Alpine, 89.7% of the 78 strong kid crop was inbred (average F = 0.0993 ± 0.0705). Seven of the nine sires were inbred (average F = 0.0799), while 31 of the 53 does had an average F coefficient of 0.0606. The maximum F was 0.3145. The rate of inbreeding coefficient (ΔF) per generation was estimated at 0.0146, 0.0857 and 0.0451 for the Saanen, Toggenburg and British Alpine respectively in 2012.

Table 4.11 Number of Saanen, Toggenburg and British Alpine kids born in 2012 grouped according to their inbreeding levels

Inbreeding level	Saanen	Toggenburg	British Alpine
0-5%	300	6	22
6-10%	64	3	17
11-15%	46	2	24
16-20%	48	6	10
21-25%	10	4	1
26-30%	17	0	1
31-35%	3	0	3
36-40%	0	0	0
41-45%	0	1	0

In Table 4.11 the inbreeding level distributions of the kids born in 2012 are presented for the Saanen, Toggenburg and British Alpine populations. The maximum inbreeding level was observed in the Toggenburg, where one kid fell into the 41-45% inbreeding level class. The Saanen and the British Alpine each had three kids in the 31-35% inbreeding level category.

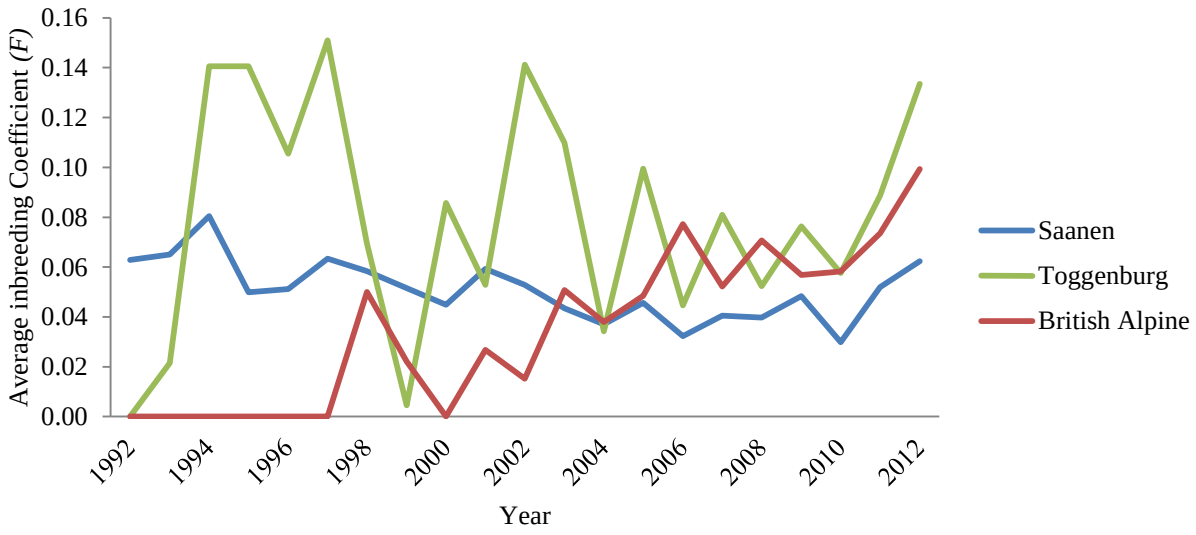


Figure 4.9 Average inbreeding coefficients (*F*) of the Saanen, Toggenburg and British Alpine kids registered between 1992 and 2012

The effective population sizes (N_e) of the Saanen, Toggenburg and British Alpine populations are shown in Figure 4.10. It firstly was calculated by considering the number of breeding animals in the previous generation interval (Method 1 – solid lines), and secondly by considering the rate of change of the inbreeding

coefficient (ΔF) (Method 2). This method is normally considered more accurate, but the first method is preferred when data is missing, although it may overestimate the true N_e (Groeneveld *et al.*, 2010).

As seen from Figure 4.10, the N_e of the Saanen, Toggenburg and British Alpine populations could not be calculated in several different years using ΔF due to lack of data. In 2009 Method 2 furthermore estimated that the N_e of the Saanen was 1667 animals (data point not shown in Figure 4.10). This method estimated that in 2012 the N_e of the Saanen was 36 animals; while the Toggenburg was 18 animals and the British Alpine were 13.

Due to the lack of data seen in several different years, Method 1 - using the number of breeding animals in the previous generation interval - was also used to calculate the effective population size. Method 1 estimated that the N_e of the Saanen was 341, while the Toggenburg was 63 and the British Alpine 53 in 2012. A large discrepancy was therefore found between the results obtained using ΔF to calculate the effective population size (Method 2) and Method 1 - using the number of parents in the previous generation interval.

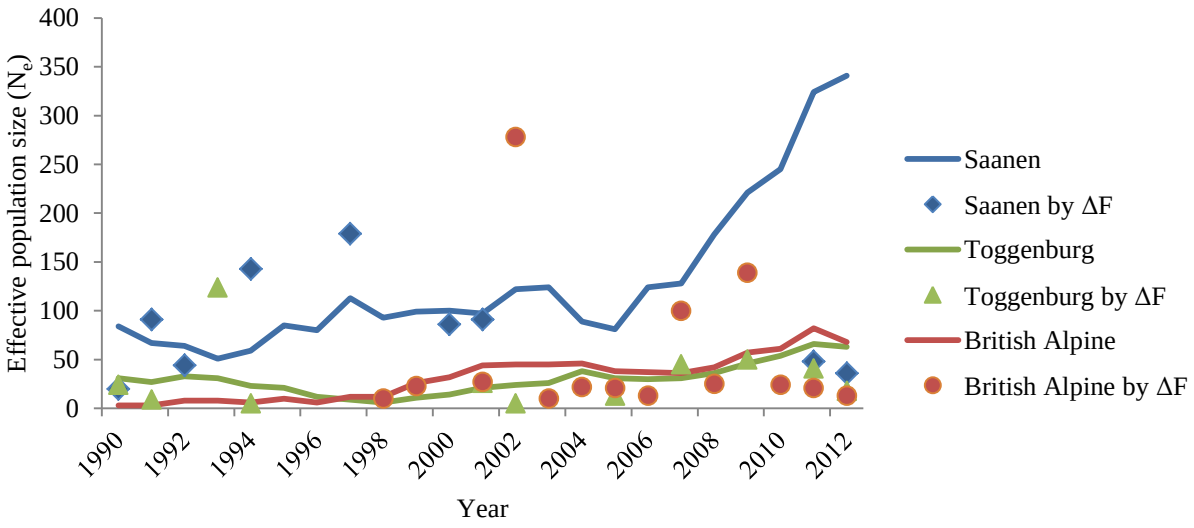


Figure 4.10 Comparison of the effective population size (N_e) estimates of the Saanen, Toggenburg and British Alpine populations when estimated by the number of parents within one generation interval (solid line) and by the rate of change in the inbreeding coefficient (ΔF) (markers)

4.6 Phenotypic anomaly

It has been observed by breeders that Saanen does mated to Saanen bucks sometimes give birth to twins with dissimilar colour patterns. The one kid would display the typical Saanen white coat, while the other would have the black coat and Swiss markings of a British Alpine. It was then reported that a Saanen doe on the University of Pretoria (UP) Experimental Farm kidded twins in August 2013, of which the one

kid phenotypically resembled a British Alpine (Figure 4.11 a). This was considered relevant to this study due to the STRUCTURE results found, and is therefore included in the results.

The UP Experimental Farm's herd of dairy goats consists solely of Saanen, and is managed as a commercial herd. Commercial Saanen bucks are used, which are sourced through local breeders, and are replaced in a predetermined cycle. During this study, the sire of the British Alpine kid born at the UP Experimental Farm was also genotyped. The dam did not fall into the random sampling group, and was therefore not genotyped. Both of the parents conform phenotypically to Saanen standards (Figure 4.11 b and c), and the sire can be seen in expanded STUCTURE bar plot in Addendum F – denoted as individual 120 – to fall in Saanen Cluster 1, although he does share a proportion of his genotype (± 6%) with the Toggenburg Cluster.

a)  b)  c) 

Figure 4.11 (a) Twin kids born on the UP Experimental Farm from a Saanen ♀ x Saanen ♂ mating, (b) Saanen dam and (c) Saanen sire of the kid with a British Alpine colour pattern

The occurrence of a kid with a British Alpine colour pattern can partly be explained through colour genetics, as the black coat with Swiss marking is recessive to the completely white patterns seen in the Saanen (Adalsteinsson *et al.*, 1994). Phenotypically this kid resembles a purebred British Alpine kid, and if its parentage was unknown, would be eligible for registration as a British Alpine. This incident partly explains why some of the individuals sampled during this study - both registered and unregistered animals – clusters with a breed not their own. This incident and its implications will be discussed in further detail in Chapter 5.

Chapter 5

Discussion

5.1 Introduction

The FAO/ISAG advisory panel recommends that a minimum of 25 animals per breed should be typed when conducting diversity studies (FAO, 2011), but also recommends that more animals should be typed when populations are small in order to capture most of the diversity in the population, and to determine any population subdivision. A total of 240 commercial dairy goats, comprising of 130 Saanen, 51 Toggenburg and 59 British Alpine goats were genotyped with 25 microsatellite markers. All of the markers amplified successfully, and the genotypic data generated were analysed with various statistical software to determine the diversity within and among breeds and to visualise the population structure of these three breeds.

Furthermore pedigree data collected since the mid 1900's on the three breeds were obtained from SA Stud Book. The pedigrees were then analysed using the POPREPORT software (Groeneveld *et al.*, 2010) to determine the generation intervals, breeding ages, family sizes, pedigree completeness, levels of inbreeding in the respective populations, as well as the effective population sizes (N_e) of the Saanen, Toggenburg and British Alpine. In this chapter the survey results will also be discussed, along with the phenotypic anomaly that was discovered during the study.

5.2 Survey discussion

The voluntary survey performed in this study indicated that only two breeders (≈ 25%) had herds of more than 400 goats. The smallest herd consisted of 47 animals. Muller (2005) reported that more or less 80% of the breeders in South Africa has smallish herds (defined as less than 60 does), which is consistent with the numbers found from the voluntary survey. The average dairy goat breeder in France has a herd of 145 animals (Danchin-Burge *et al.*, 2012).

It was also found that alternative breeding technologies such as artificial insemination (AI) are rarely used by South African dairy goat breeders - three of the nine respondents indicated limited use, instead making use of natural service to a greater degree. In comparison it was found in the French dairy goat breeding system that although the overall use of AI in the national herd was around 9% (Danchin-Burge *et al.*, 2012), the use of AI in the nucleus herds could be as high as 40%. In South African dairy cattle between 25 and 36% of the progeny born in the period between 2000 and 2003 were the offspring of foreign AI sires (Maiwashe *et al.*, 2006) which illustrates the extensive use of AI in the national dairy cow herd. Increasing the use of AI in the South African dairy goat populations, especially that of foreign sires, would improve the genetic diversity in the South African populations, as well as provide some linkage with foreign populations. Caution should however be exercised to prevent the overuse of a popular sire, as it may result in increased inbreeding levels in the South African population (Maiwashe *et al.*, 2006).

Most of the dairy goat breeders in South Africa breed their own replacement bucks and does, and sometimes may obtain stock from a local co-breeder. While some use criteria to select their replacement stock, the buck is often only seen as a means to get the doe pregnant and back into production (Muller, 2005). The genetic effect of the buck on the herd is estimated to be around 87% (Olivier *et al.*, 2005), as his daughters are very likely to become replacement does themselves. The practice of using own-bred replacement bucks in one herd decreases the genetic linkage between herds (Muller, 2005) and may also decrease the diversity within herd itself. A couple of the breeders indicated that they routinely practise crossbreeding to combat the loss in diversity and increase the milk solids. This practise is also used by dairy cattle breeders (Boettcher, 2001), and similarly dairy goat breeders would need a well thought-out breeding plan to maintain the levels of heterosis after the F_1 generation.

In France 90% of the goat milk that is produced is processed to be sold as cheese (Dubeuf *et al.*, 2004; Danchin-Burge *et al.*, 2012), while in South Africa it was seen from the survey that fresh milk and yogurt are also products that producers feel are worthwhile marketing, along with hard and soft cheese varieties. A couple of producers used goats' milk to make kefir and soap. The French breeding programme's main goal is the improvement of the population's protein yield (Danchin-Burge *et al.*, 2012) due to the focus on cheese production. Brazil, as an example of a developing country, has a greater focus on the total milk yield (Lopes *et al.*, 2012). The South African dairy goat industry lacks the directed breeding programmes seen in these two countries, and individual breeders select according to their production goals; the efficiency of selection may be questionable when considering that the numbers of breeders that do take part in the official Milk Recording Scheme are in the minority. The differences in the marketing of the goats' milk products between the French and the South African industries are also marked – the French cheeses are protected designation of origin (PDO) and marketed through official channels (Danchin-Burge *et al.*, 2012). The South African goats' milk products in contrast are more often marketed to industry or processed by the producer himself, and then marketed through on-farm sales or through informal markets.

5.3 Genetic characterization

The genetic characterization of the South African Saanen, Toggenburg and British Alpine populations based on the 25 microsatellite markers revealed a moderate genetic diversity. The polymorphic information content (PIC) of a panel is considered to be highly informative if the mean PIC is above 0.50 (Tolone *et al.*, 2012). The PIC value of the 25 markers in the Saanen breed averaged 0.60, and was similar to that found in the Toggenburg and British Alpine. The range of the PIC values in the Toggenburg and the British Alpine were greater than observed in the Saanen. An average PIC of 0.67 was seen for six Portuguese goat breeds based on 25 microsatellites, similar to those achieved for this study (Bruno-de-Sousa *et al.*, 2011) which had 11 markers in common with the current study. Sixteen of the markers used in this study were chosen from the FAO/ISAG list of recommended microsatellites for diversity studies. These markers are recommended

based on their polymorphicity in various goat breeds; therefore it was expected that the panel of microsatellites used for this study would be at least moderately polymorphic.

The 25 microsatellite markers were also tested for deviation from Hardy Weinberg Equilibrium (HWE) within each population. In the Saanen, it was found that 20 of the 25 loci were in Hardy Weinberg equilibrium ($P > 0.05$), while the Toggenburg and British Alpine had 19 loci that were in HWE. Only one locus (INRA40) was found to deviate in all three breeds. None of these markers deviated to such a degree that it had to be discarded, as in the study of the Sicilian sheep breeds (Tolone *et al.*, 2012).

The mean number of alleles (MNA) across the Saanen, Toggenburg and British Alpine populations ranged from 3 to 12 (average 8), and compares well with Iamartino *et al.* (2005), where an MNA of 7.3 was found across nine different breeds, including the Saanen and Alpine. Glowatzki-Mullis *et al.* (2008) conducted a study using mostly Swiss breeds, and found an MNA of 9.6, which was higher than the observed MNA in this study. The MNA of the Saanen observed in this study (6.8) compared favourably to that of the Swiss population used by Glowatzki-Mullis *et al.* (2008), where an average of 5.1 alleles were observed per locus. It was however lower than the observed MNA of 7.3 seen in the Italian Saanen population (Iamartino *et al.*, 2005). The MNA of the British Alpine (6.84) was also lower in comparison to MNA of 7.1 seen in the Italian Alpine population (Iamartino *et al.*, 2005), while the MNA observed in the Toggenburg population was higher than that seen in the Swiss Toggenburg population (6.44 versus 5.1) (Glowatzki-Mullis *et al.*, 2008). The allelic diversity observed in these populations were consistent with the results obtained in similar studies.

The overall genetic diversity was moderate in all three breeds, varying from 62.6% to 63.4%. This was similar to results seen for the Swiss Saanen and Toggenburg populations (Glowatzki-Mullis *et al.*, 2008) where the Saanen had a slightly higher H_O than H_E (0.60 versus 0.59), and the Toggenburg's H_O and H_E were both equal to 0.59. Bruno-de-Sousa *et al.* (2011) used 25 microsatellites to genotype six Portuguese goat breeds, and found that the mean observed heterozygosity was slightly lower than the expected heterozygosity. The differences between the observed and expected heterozygosities for the Saanen, Toggenburg and the British Alpines observed in the current study were small. These small differences indicate that these populations are largely in balance, and that no significant loss of heterozygosity has occurred (Falconer, 1989).

It was also interesting to note that 33 private alleles were identified in the sampled Saanen, Toggenburg and British Alpine populations, but that only four of the 33 private alleles had a frequency greater than 1% in the Saanen and British Alpine populations (two private alleles each). The Toggenburg did not have any private alleles that occurred more frequently than in 0.5% of the population. The highest private allele frequency observed was 0.036 in the Saanen (allele 215 of INRA23). This was low in comparison to the frequency of the private allele identified for the Swiss Toggenburg (0.11) in the study by Glowatzki-Mullis *et al.* (2008). A private allele is considered to have a high frequency if it occurs in more than 20% of the population (Glowatzki-Mullis *et al.*, 2008).

In the analysis of Wright's F_{IS}-values, it was found that three markers had a significant deficiency of heterozygotes in the Saanen breed, but that none of these markers were found on the same chromosome. Only INRA23 (CHI3) had a significant heterozygote deficiency in the British Alpine, while no significant heterozygote deficiency in any of the markers was seen in the Toggenburg. The low negative F_{IS} values observed for these breeds, combined with the low F_{IT} values indicate very limited inbreeding in the respective breeds (Tolone *et al.*, 2012). The average F_{IT} and F_{IS} values found in this study were lower than those found for the five Sicilian sheep breeds (0.08 and 0.03) by Tolone *et al.* (2012). Much higher F_{IS} and F_{IT} values – 0.18 and 0.32 - were found for the Indian goat breeds (Dixit *et al.*, 2012), which indicated slightly higher levels of inbreeding in the populations studied.

The F_{ST} value of the Saanen was found to be 0.05. The low positive F_{ST} value indicates that the genetic differences between the different Saanen herds included in this study are very slight, which is consistent with the common ancestry of the Saanen goats in South Africa. The same inference can be made about the Toggenburg and British Alpine populations included in this study, as the F_{ST} value in the Toggenburg and British Alpine breeds were very similar to that seen in the Saanen (0.053 and 0.052 respectively). Tolone *et al.* (2012) found a mean F_{ST} of 0.05 for the five Sicilian sheep breeds, and concluded also that the differentiation between the breeds were very slight, and that these breeds probably shared a common history and similar breeding practices. This is probably also true for the three dairy goat breeds in this study, as all three originate from Swiss stock, and had undergone selection for milk production traits. In contrast the F_{ST} value found for the Indian goat breeds were somewhat higher at 0.17, which indicated that the breeds differentiated at a genetic level and did not just differ in phenotype (Dixit *et al.*, 2012).

Wright's *F*-statistics were obtained for all three breeds over all the loci, in order to determine the relationship between the breeds. Twelve of the 25 markers had negative F_{IS} values, while thirteen had low positive values. The low positive average F_{IS} value (0.020) that was observed confirms the trend seen when the three breeds are considered separately – each of the three previous results' negative average F_{IS} value indicated that very limited inbreeding occurred within the populations. The F_{ST} value (0.064) is very similar to the values found for the Saanen, Toggenburg and British Alpine in the previous section (0.050, 0.053 and 0.52 respectively). These values are also similar to those found for the Sicilian sheep breeds (Tolone *et al.*, 2012). While it was inferred that there is very little genetic difference within the Saanen, Toggenburg and British Alpine populations, it is also now shown that there is very little genetic difference among the three breeds as well. This is expected since the Saanen, Toggenburg and British Alpine breeds have all been developed as dairy goats, and therefore are expected to have a large number of traits in common. These results were further confirmed when an AMOVA was performed, which indicated that most of the variation seen is due to the differences in the individuals themselves (91.7%), while 6.4% of the variation is due differentiation between breeds. A small amount of differentiation (1.9%) is due to the breed effect within populations.

5.4 Population structure

The genotypic data of the three breeds – Saanen, Toggenburg and British Alpine – were analysed with STRUCTURE, and it was found that the most appropriate population number was six. This result was in contrast to the expectation when analysing three breeds. Bruno-de-Sousa *et al.* (2011) conducted a similar analysis with six Portuguese goat breeds, and found that the most appropriate number of populations were equal to the number of breeds analysed. Glowatzki-Mullis *et al.* (2008) in contrast found less populations than the number of breeds analysed (9 populations versus 11 breeds), and concluded that the three breed clustering in the same population shared a common ancestry, and had not yet differentiated enough to form their own separate clusters.

It was observed in this study that the three breeds did, for the most part, cluster together as expected when only three populations were considered. When six populations were considered however, it was observed that the Toggenburg formed a single cluster, although its membership has dropped to 72.8%, while most of the British Alpine grouped together in cluster 1 (59.6%). The Saanen breed clustered into 3 distinct groups (21.6%, 35.6% and 31.0% of the population). This is indicative of some genetic differentiation taking place within the Saanen breed (Glowatzki-Mullis *et al.*, 2008; Bruno-de-Sousa *et al.*, 2011). In the previous section it was established that the average inbreeding of the Saanen breed was low, based on the average F_{IS} value of -0.005. Although several different herds from several geographical locations were sampled, no clear geographical influence was seen that could be attributed to this clustering pattern. In Saanen 2 for instance, two of the main contributors to this cluster were from the Western Cape and Limpopo, which are at opposite ends of the country. Saanen 1 consisted of goats mainly from the Western Cape and Gauteng, while Saanen 3 had goats from Limpopo, KwaZulu Natal and the Western Cape. However, when the breeders' practice of breeding their own replacement bucks is taken into account (Muller, 2005), it becomes probable that at some point in the past the herds in the same clusters obtained bucks from the same source. Due to mostly breeding their own replacement bucks, the genetic influence of the ancestral goats became amplified in these herds, causing some inbreeding which in turn caused these populations to differentiate to the point that they formed their own clusters.

Another unexpected differentiated population was observed in cluster 3. Cluster 3 had individuals with membership from all three breeds, consisting mainly of British Alpine and Toggenburg individuals, with a smaller number of Saanen individuals. From the 29 individuals seen in cluster 3, 12 originate from Farm H, while another six, five and four were sampled from Farms M, B and I respectively. Glowatzki-Mullis *et al.* (2008) observed a similar situation where the Tessin Grey goat, Nera Verzasca goat and the Peacock goat breeds all grouped together in the same cluster. It was concluded that because of their similar geographical origin and breed history that some admixture may have occurred that caused these populations to be genetically similar. It may be therefore assumed that there has been some admixture between the goats found in cluster 3, which then differentiated them from their parent Saanen, Toggenburg and British Alpine populations. This cluster could therefore be considered as a crossbred population. This cluster is problematic,

as at least one of the goats found in this cluster are registered as a purebred animal which does not cluster within its own purebred cluster.

5.5 Pedigree analysis

The generation interval for the South African populations of the Saanen, Toggenburg and British Alpine (3.4, 3.9 and 3.2 years respectively) in this study was similar to the generation intervals reported for the French Saanen and Alpine breeds (4.0 and 4.1 years) (Danchin-Burge *et al.*, 2012). Only 8.2% of the recorded Saanen does completed three or more lactations while 7.5% in the Toggenburg and 13.1% in the British Alpine does managed three completed lactations. As the highest production for the dairy goat is normally seen during parity three or four (Goetsch *et al.*, 2011), these figures suggest that the dairy goat population in South Africa is performing below their capacity, and are in fact leaving the herd too soon. The small family sizes of the South African does (1.6 – 1.9) could be attributed to the does leaving the herd too soon, therefore not contributing large numbers of offspring to the next generation. The small doe family sizes could also be due to the large number of young does that entered production in the last two years. These does would not have had time to have more kids than biologically possible in that timeframe, and could therefore have skewed the resultant family size estimates.

The official pedigree recording of the Saanen, Toggenburg and British Alpine populations started in the same time period as that of the South African dairy cattle breeds, namely the Holstein, Jersey, Ayrshire and Guernsey breeds (Maiwashe *et al.*, 2006). The number of dairy goat pedigree records available over a similar time period is vastly outnumbered by those of dairy cattle though (4013 Saanen vs. 890 598 Holstein records). The pedigree completeness of the dairy goat breeds (71%, 73% and 83% for the Saanen, Toggenburg and British Alpine respectively) was similar to that seen in the dairy cattle breeds, where the Guernsey breed had 70% pedigree completeness over its recording period, versus the Jersey breed with 90% completeness (Maiwashe *et al.*, 2006). The PCI is a measurement of the reliability of inbreeding values. The algorithm used by POPREPORT assumes that animals with unknown parents are unrelated to the overall population, and allocates an inbreeding coefficient of zero (Mucha & Windig, 2009), which may lead to an underestimation of the true inbreeding levels in a population. This is of special importance in the South African commercial dairy goat population, where the three herdbooks are open, and very few of the animals are registered with complete pedigree information.

The Toggenburg had the highest rate of inbreeding change per generation (ΔF) when compared to the British Alpine and the Saanen. The Toggenburg ΔF of 0.0857 (8.57%) and British Alpine ΔF of 0.0451 (4.51%) far exceeds the FAO guidelines of a ΔF not exceeding 0.01 (1%) per generation (Mucha & Windig, 2009). These levels were also higher than those seen in the South African dairy cattle breeds, which varied from 0.05% to 0.07% (Maiwashe *et al.*, 2006); Canadian Holsteins and Jerseys had a ΔF of 0.014% and 0.011% respectively (Stachowicz *et al.*, 2011). The average inbreeding coefficient seen in the French Saanen population is 2.21% (Danchin-Burge *et al.*, 2012), compared to the South African Saanen which was found

to be 6.23%. The high rate of inbreeding change seen in the South African dairy goat breeds is likely due to the increased interest and demand for these animals. Registrations of all three breeds have increased in the last decade, with limited opportunity for new genetic stock, which may have contributed to the increased levels of inbreeding observed in this study.

There is a disjunction between the inbreeding results obtained with the pedigree analysis and the genetic analysis; it was observed that lower estimates were obtained for all three breeds in the genetic analysis. This could possibly be explained by the fact that only the goats present in the pedigree file could be used in the calculation of the inbreeding coefficient for the breeds during the pedigree analysis. Normally only stud goats are registered, and as such a closer relationship between these animals are expected. During the sampling for the genetic component of the diversity study a conscious effort was made to sample as widely as possible without sampling related animals. Therefore both registered and grade animals were included in the genetic component. Relationship data on the grade animals would not be available for inclusion in the pedigree analysis, and was therefore not considered in the calculation of the inbreeding coefficient during that analysis. It is possible then that these results are not as accurate as it would have been if all the animals in the population were included in the analysis. It is furthermore not clear whether an over- or underestimation has been performed on the inbreeding values and the rate of change. While the values might decrease if more animals are considered, it may also increase because the small populations in South Africa originated from a small foundation population, and grade animals are therefore conceivably related to the registered animals.

The effective population size (N_e) of the Saanen, Toggenburg and British Alpine populations was 341, 63 and 53 respectively when estimated by considering the number of parents in the previous generation interval (Method 1). When calculating the N_e according to the ΔF over the years (Method 2) however, the population sizes were 36, 18 and 13. The discrepancy observed between the results obtained with the two methods used to calculate the N_e could firstly be explained by lack of data. Due to the number of animals that are added to the herdbooks with unknown parents, which are assumed to be unrelated to the population and therefore have no inbreeding coefficients, the average inbreeding of the populations becomes skewed. It was seen that the N_e could not be calculated for several years, as the skewed mean F of the population caused ΔF to be equal or less than zero. To counter the lack of historical data a base year is normally assigned for the calculation of ΔF and therefore N_e (Groeneveld *et al.*, 2010); alternatively animals with incomplete records can be removed from the data set. In the case of the Saanen, Toggenburg and British Alpine pedigrees these options were unfeasible due to the fluctuations seen in the completeness of the pedigree records throughout the years, and removing animals from an already small dataset would not give a trustworthy N_e.

It is known that using the number of parents in the previous generation interval to estimate the N_e furthermore gives an overestimation of the N_e if the male:female ratio in a population is more than 1:1 (Falconer, 1989; Groeneveld *et al.*, 2010). In the dairy goat population the bucks are usually outnumbered by the does, and because they are in the minority they have a greater impact on the N_e calculation. It is therefore

probable that the true N_e is much lower than the results found using this method. It also cannot be assumed that the results found by using ΔF are the true N_e for the Saanen, Toggenburg and British Alpine populations. The true N_e of these populations most likely lie somewhere between the two estimates, but lack of data prevents the calculation of a more accurate estimate.

The dairy cattle breeds in South Africa (Holstein, Jersey, Guernsey and Ayrshire), despite having much larger populations in comparison to the dairy goats, had an N_e that varied between 108 and 165 (Maiwashe *et al.*, 2006). In the study by Danchin-Burge *et al.* (2012) it was found that the N_e of the French Saanen population varied between 149 and 203 animals, while the Alpine population was between 129 and 169 goats. It was also found that the N_e of these breeds were above the FAO recommendation to maintain an effective population size of 50 – 100 animals within a breed (Mucha & Windig, 2009; Danchin-Burge *et al.*, 2012). In comparison to the results obtained in the French study, the Saanen with an estimated N_e between 341 and 36 animals either falls above or below the FAO recommendation (FAO, 1998). Similarly the Toggenburg (N_e between 63 and 18) and the British Alpine (N_e between 53 and 13) falls either just within the FAO minimum N_e recommendation or below it. If only the lowest N_e estimates are considered, then according to the threat status criteria set out in Table 2.7 the South African Saanen, Toggenburg and British Alpine populations are critical as they have an effective population size of less than 50 animals each. Steps need to be taken urgently to increase the diversity in these populations.

5.6 Phenotypic anomaly

The incidence of black kids being born from a Saanen ♀ x Saanen ♂ mating has been mentioned both by breeders and observed at the University of Pretoria (UP) Experimental Farm. In the incident recorded in this dissertation a female black kid with Swiss markings was born from such a mating, and was a twin to a male white goat. According to Adalsteinsson *et al.* (1994) such a colour discrepancy is possible because the black colour is recessive to the complete white pattern seen in the Saanen. However both of the parents should be carriers of the black colour in order to produce non-white offspring. The complete white pattern is a characteristic of the Saanen breed, and is dominant over all other colour patterns such as the brown with Swiss markings of the Toggenburg and the black with Swiss markings of the British Alpine. A goat that is homozygous recessive would be completely black with no pattern (Adalsteinsson *et al.*, 1994).

From these colour inheritance principles it is therefore possible that the offspring of a Saanen x British Alpine or Toggenburg mating would have the complete white pattern of its Saanen parent due to the dominance of the white pattern. If the offspring remained in a Saanen herd, the black allele could be masked indefinitely through the successive generations if mated to pure Saanen goats. Should the carrier goat be mated to another carrier though, 25% of the offspring should display the recessive colour pattern instead of the Saanen white pattern.

The above scenario does present problems in the South African dairy goat registration system. As an effort is being made to keep the breeds pure by the SAMGBS while trying to increase the number registered

goats, goats with unknown parents may be added to the relevant herdbook based on an inspection. A major factor in assigning a goat to a breed lies in its colour pattern. Therefore it might be possible to register a black goat with Swiss markings and unknown parents as a British Alpine. It could also be possible that such a goat may be the offspring of a Saanen x Saanen mating as observed on the Experimental Farm, and as such probably share more of its genetic make-up with the Saanen population than with the British Alpine population.

This scenario is also a possible explanation for the results seen in the STRUCTURE analysis. It was observed that several goats clustered with breeds not their own, and either one of their parents or they themselves might have been assigned to the wrong breed during an inspection based on their coat colour. At the same time this scenario may be the origin of the crossbred population seen in cluster 3. The ancestors of the goats in this cluster may have been believed to be of one breed while actually sharing their DNA with another, and inadvertently was used to crossbreed in their population, thereby causing cluster 3 to differentiate from the Saanen, Toggenburg and British Alpine populations.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

In this study the South African dairy goat breeds commonly used by commercial producers – the Saanen, Toggenburg and British Alpine - were characterised by 25 microsatellite markers. From the genotypic analysis it was found that none of the three populations had a significant deficit of heterozygotes, and none deviated significantly from Hardy-Weinberg equilibrium. Through a population structure analysis it was found that the Saanen differentiated into three sub-populations, while the Toggenburg and the British Alpine each segregated into their own clusters. A crossbred population cluster was also identified, in which mainly Toggenburg and British Alpine goats were found. Another significant, though unintended, part of this study was the practical implications of goat coat colour inheritance. This explained some of the inconsistent results seen during this study, such as where goats of one breed clustered with another breed rather than with their own

A pedigree analysis was also done for these breeds, based on the pedigree records collected from the mid-1990's. A major challenge identified through this analysis was the completeness of the records, as animals with unknown parents could be added to the herdbooks based on an inspection. This complicated the determination of parameters such as the effective population size, as missing data would lower the accuracy of the estimations. It was found that the true N_e of the Saanen probably lay somewhere between 341 and 36 animals, between 63 and 18 for the Toggenburg and between 53 and 13 in the British Alpine.

This study can be considered as a benchmark for dairy goats in South Africa, as these breeds have never been characterised on a molecular level before. The ability of the dairy goat population to respond to selection pressure and increase their productivity is subject to the amount of genetic diversity found within these breeds (Boettcher, 2001). Healthy and productive animals will increase the profits of the breeders, as well as satisfy the consumer's concerns about the production process (Boettcher, 2001; Barillet, 2007). Maintenance of this diversity within the Saanen, Toggenburg and British Alpine breeds is necessary to prevent excessive inbreeding in these populations. The rate of inbreeding of the registered populations was calculated as 1.46%, 4.51% and 8.57% for the Saanen, British Alpine and Toggenburg breeds, which exceeds the FAO recommendations of no more than 1% inbreeding per generation (Mucha & Windig, 2009).

6.2 Recommendations

Currently the SAMGBS allows the registration of goats with unknown parents with the relevant herdbook based on an inspection. It was however found during this study that the true identity of a goat may be masked through colour inheritance principles, and as such may be assigned to the wrong breed based on coat colour alone. It is instead recommended that goats with unknown parents rather be added to an appendix herdbook to prevent the dilution of data in the Herdbook Proper. As laid out by the SA Stud Book

constitution (http://studbook.co.za/Constitution/Page_21-23.pdf), the offspring of animals falling into an Appendix A herdbook could be upgraded to the Appendix B herdbook if mated to a sire either from the Herdbook Proper or Appendix B. In turn the offspring of an Appendix B dam would be upgraded to the herdbook proper if mated to a sire from the herdbook proper. This system would ensure that the animals added to the Herdbook Proper share at least 75% their genetic make-up with other animals in the herdbook, and gives the opportunity for appendix animals to take part in recording schemes without diluting the results of the purebred goats. In the French nucleus herds a goat may not have more than 6.25% foreign genes to qualify as a purebred (Danchin-Burge *et al.*, 2012).

Evaluation of the dairy goat populations are also difficult as there is very little traceable genetic linkage between the herds (Muller, 2005) which is compounded by poor recordkeeping. There is a tendency among breeders to breed their own replacement bucks and does, and the effect of the buck on the herd is being underestimated (Olivier *et al.*, 2005). There is also no progeny testing of bucks in South Africa, and the use of AI in the dairy goat industry is minimal (Muller, 2005). There are several difficulties associated with importing live goats to South Africa, which makes AI from foreign sires a more viable option to introduce new blood to the South African population. It is recommended that a nucleus herd be kept which would be able to supply high potential bucks to breeders. These bucks should be progeny tested to confirm their potential. This would also allow for better genetic linkages between the herds, which in turn would make a BLUP prediction of the population more viable and accurate. At the same time it would address the recordkeeping practices in the industry.

This study has also displayed the usefulness of DNA-based techniques in eliminating any bias that may be found due to inaccurate recordkeeping. The practice of over-mating in the commercial system makes it difficult to identify specific sires, which then causes the pedigree of the offspring to be incomplete. This problem can be overcome by using DNA-based parentage testing to determine the most probable sire of the offspring. The parentage panel developed by Visser *et al.* (2011b) for the South African Angora goat was also verified in a Saanen sub-population (Friedrich, 2009). Thirteen of the fourteen recommended microsatellite markers in this panel were in common with this study, and these markers amplified well in the genotyped Saanen, Toggenburg and British Alpine populations. This parentage panel should therefore be suitable for use in the commercial dairy goat population to determine the parentage of the offspring and to improve recordkeeping practices.

Accurate estimation of the population status of these breeds in South Africa is near impossible due to poor recordkeeping. It was also observed that only a fraction of the productive population takes part in the official milk recording scheme, and much information is lost through this. Incentivizing the breeders through offering a premium for breeding stock with accurate breeding values may help to improve the recording of pedigrees. Offering a premium for milk with high butterfat or large volumes of milk may encourage participation in the milk recording scheme, but with no central marketing chain which would offer this,

participation will remain poor. It would be up to the breeders to reach a consensus on marketing strategies, and therefore breeding strategies to improve production of goat milk in the face of growing demand.

This study was the first to characterize the South African commercial dairy goat breeds by using molecular techniques. It is therefore recommended that the evaluation of the Saanen, Toggenburg and British Alpine breeds should be repeated every ten years to monitor the change in the genetic diversity of these breeds, to enable corrective measures to be implemented timeously should the genetic diversity decrease.

References

Adalsteinsson, S., Sponenberg, D.P., Alexeiva, S. & Russel, A.J., 1994. Inheritance of goat coat colors. J. Hered. 85, 267-272.

Agriculture and Agri-Food Canada, 2006. Canadian dairy goat industry profile. Agriculture and Agri-Food Canada, Canada. http://www4.agr.gc.ca/resources/prod/doc/dairy/pdf/goatprofile_e.pdf accessed on 19 April 2014.

Ahuya, C.O., Ojango, J.M.K., Mosi, R.O., Peacock, C.P. & Okeyo, A.M., 2009. Performance of Toggenburg dairy goats in smallholder production systems of the eastern highlands of Kenya. Small Ruminant Res. 83, 7-13.

Al-Shorepy, S.A., Alhadrami, G.A. & El Awad, A.I., 2010. Development of sheep and goat production system based on the use of salt-tolerant plants and marginal resources in the United Arab Emirates. Small Ruminant Res. 91, 39-46.

Al-Tamimi, H.J., Obeidat, B.S., Abdullah, A.Y. & Atiyat, R.M., 2013. Disproportionate thermophysiological strain between intensively- and extensively-managed goats during summer. Small Ruminant Res. 109, 1-8.

Alexandre, G. & Mandonnet, N., 2005. Goat meat production in harsh environments. Small Ruminant Res. 60, 53-66.

Barillet, F., 2007. Genetic improvement for dairy production in sheep and goats. Small Ruminant Res. 70, 60-75.

Barrera-Saldaña, H.A., Ascacio-Martínez, J.A., Sifuentes-Rincón, A.M., Arellano-Vera, W. & Arbiza, S.I., 2010. Applications of biotechnology and genomics in goats. Small Ruminant Res. 89, 81-90.

Baumung, R., Simianer, H. & Hoffman, I., 2004. Genetic diversity in farm animals - a survey. J. Anim. Breed. Genet. 121, 361-373.

Beuzen, N.D., Stear, M.J. & Chang, K.C., 2000. Molecular markers and their use in animal breeding. Vet. J. 160, 42-52.

Bhargava, A. & Fuentes, F.F., 2010. Mutational dynamics of microsatellites. Mol. Biotechnol. 44, 250-266.

Bodó, I., 1989. Methods and experiences with in situ preservation of farm animals. In: Animal genetic resources: a global programme for sustainable development. FAO, Rome, Italy. pp 85-102. URL: ftp://ftp.fao.org/docrep/fao/009/t0284e/T0284E00.pdf.

Boettcher, P.J., 2001. 2020 Vision? The future of dairy cattle breeding from an academic perspective. J. Dairy Sci. 84, Supplement, E62-E68.

Bourdon, R.M., 2000. Genetic predictions. In: Understanding animal breeding. 2nd ed. Prentice-Hall, Inc., Upper Saddle River, New Jersey 07458. pp. 245-254.

Boyazoglu, J., Hatziminaoglou, I. & Morand-Fehr, P., 2005. The role of the goat in society: Past, present and perspectives for the future. Small Ruminant Res. 60, 13-23.

Bruno-de-Sousa, C., Martinez, A.M., Ginja, C., Santos-Silva, F., Carolino, M.I., Delgado, J.V. & Gama, L.T., 2011. Genetic diversity and population structure in Portuguese goat breeds. Livest. Sci. 135, 131-139.

Buduram, P., 2004. Genetic characterisation of Southern African sheep breeds using molecular markers. MSc Thesis. Department of Animal, Wildlife and Grassland Sciences,, University of the Free State. Bloemfontein.

Burt, D.W., 2009. The cattle genome reveals its secrets. J. Biol. 8, 36.

Buschiazzo, E. & Gemmell, N.J., 2006. The rise, fall and renaissance of microsatellites in eukaryotic genomes. BioEssays. 28, 1040-1150.

Caballero, A., 1994. Developments in the prediction of effective population size. Heredity. 73, 657-679.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B. & Pang, E.C.K., 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica. 142, 169-196.

Danchin-Burge, C., Allain, D., Clément, V., Piacère, A., Martin, P. & Palhière, I., 2012. Genetic variability and French breeding programs of three goat breeds under selection. Small Ruminant Res. 108, 36-44.

Department of Government Communciations and Information Systems, 2012. Agriculture, Forestry and Fisheries. In: South Africa Yearbook 2012/13. Government Communications and Information Systems, Republic of South Africa. pp 56-58. URL: http://www.gcis.gov.za/content/resourcecentre/sa-info/yearbook2012-13. 15 January 2014.

Directorate: Agricultural Information Services, 2003. Mad cow disease - BSE - Is it a real threat? Department of Agriculture, Pretoria, South Africa. http://www.nda.agric.za/docs/Infopaks/BSE.htm accessed on 19 September 2013.

Directorate: Animal Production, 2007. Status of the South African goat industry. Department of Agriculture, S.A., Com Mark Trust & National Agricultural Marketing Council, Pretoria, South Africa.

Directorate: Marketing, 2010. A profile of the South African Mohair market value chain. Department of Agriculture Forestry and Fisheries, Pretoria, South Africa.

Directorate: Marketing, 2012. A profile of the South African goat market value chain. Department of Agriculture Forestry and Fisheries, Pretoria, South Africa.

Dixit, S.P., Verma, N.K., Aggarwal, R.A.K., Vyas, M.K., Rana, J. & Sharma, A., 2012. Genetic diversity and relationship among Indian goat breeds based on microsatellite markers. Small Ruminant Res. 105, 38-45.

Dodgson, J.B., Cheng, H.H. & Okimoto, R., 1997. DNA marker technology: a revolution in animal genetics. Poultry Sci. 76, 1108-1114.

Dong, Y., Xie, M., Jiang, Y., Xiao, N., Du, X., Zhang, W., Tosser-Klopp, G., Wang, J., Yang, S., Liang, J., Chen, W., Chen, J., Zeng, P., Hou, Y., Bian, C., Pan, S., Li, Y., Liu, X., Wang, W., Servin, B., Sayre, B., Zhu, B., Sweeney, D., Moore, R., Nie, W., Shen, Y., Zhao, R., Zhang, G., Li, J., Faraut, T.,

Womack, J., Zhang, Y., Kijas, J., Cockett, N., Xu, X., Zhao, S., Wang, J. & Wang, W., 2013. Sequencing and automated whole-genome optical mapping of the genome of a domestic goat (*Capra hircus*). Nat. Biotech. 31, 135-143.

Donkin, E.F. & Boyazgolu, P.A., 2000. Milk production from goats for households and small-scale farmers in South Africa. Department of Agriculture Forestry and Fisheries, South Africa. http://www.nda.agric.za/docs/AAPS/Articles/Goats/Dairy%20Goat/Milk.pdf

Dubeuf, J.-P., 2011. The social and environmental challenges faced by goat and small livestock local activities: Present contribution of research–development and stakes for the future. Small Ruminant Res. 98, 3-8.

Dubeuf, J.-P. & Boyazoglu, J., 2009. An international panorama of goat selection and breeds. Livest. Sci. 120, 225-231.

Dubeuf, J.P., Morand-Fehr, P. & Rubino, R., 2004. Situation, changes and future of goat industry around the world. Small Ruminant Res. 51, 165-173.

Dýrmundsson, Ó.R., 2006. Sustainability of sheep and goat production in North European countries—From the Arctic to the Alps. Small Ruminant Res. 62, 151-157.

Ellegren, H., 2004. Microsatellites: simple sequences with complex evolution. Nat. Rev. Genet. 5, 435-445.

Excoffier, L. & Lischer, H.E.L., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 10, 564-567.

Falcón de Vargas, A., 2002. The Human Genome Project and its importance in clinical medicine. Int. Congr. Ser. 1237, 3-13.

Falconer, D.S., 1989. Introduction to quantitative genetics. 3rd ed. Longman Scientific & Technical, Essex, England.

Falush, D., Stephens, M. & Pritchard, J.K., 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. Genetics. 164, 1567-1587.

FAO, 1998. Secondary guidelines for development of national farm animal genetic resources management plans: management of small populations at risk. Pages 59-83. FAO, Rome, Italy.

FAO, 2011. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines No. 9. Rome.

FAO, 2012. FAOSTAT. http://faostat3.fao.org/home/index.html#DOWNLOAD accessed on 16 January 2013; 9 December 2013.

Friedrich, H., 2009. Evaluation of microsatellite markers for parentage verification in South African Angora goats. MSc Thesis. Department of Animal and Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria. Pretoria.

Galal, S., 2005. Biodiversity in goats. Small Ruminant Res. 60, 75-81.

Glaubitz, J.C., 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol. Notes. 4, 309-310.

Glowatzki-Mullis, M.-L., Muntwyler, J., Baumle, E. & Gaillard, C., 2008. Genetic diversity measures of Swiss goat breeds as decision-making support for conservation policy. Small Ruminant Res. 74, 202-211.

Goetsch, A.L., Zeng, S.S. & Gipson, T.A., 2011. Factors affecting goat milk production and quality. Small Ruminant Res. 101, 55-63.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Groeneveld, E., van der Westhuizen, B., Maiwashe, A., Voordewind, F. & Ferraz, J.B.S., 2010. POPREP: a generic report for population management. Genet. Mol. Res. 8, 1158-1178.

Grunert, K.G., 2006. Future trends and consumer lifestyles with regard to meat consumption. Meat Sci. 74, 149-160.

Gurung, N.K. & Solaiman, S.G., 2010. Goat breeds. In: Goat science and production. Ed. Solaiman, S.G. Wiley-Blackwell, Ames, Iowa, USA. pp. 21-37.

Haenlein, G.F.W., 2004. Goat milk in human nutrition. Small Ruminant Res. 51, 155-163.

Hatziminaoglou, Y. & Boyazoglu, J., 2004. The goat in ancient civilisations: from the Fertile Crescent to the Aegean Sea. Small Ruminant Res. 51, 123-129.

Hofmeyr, H.S., 1968. Milch Goats. In: The small stock industry in South Africa. 1st ed. Ed. Hugo, W.J. Department of Agricultural Technical Services, Pretoria. pp. 192-221.

Iamartino, D., Bruzzone, A., Lanza, A., Blasi, M. & Pilla, F., 2005. Genetic diversity of Southern Italian goat populations assessed by microsatellite markers. Small Ruminant Res. 57, 249-255.

Jarne, P. & Lagoda, P.J.L., 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. 11, 424-429.

Jiménez-Gamero, I., Dorado, G., Muñoz-Serrano, A., Analla, M. & Alonso-Moraga, A., 2006. DNA microsatellites to ascertain pedigree-recorded information in a selecting nucleus of Murciano-Granadina dairy goats. Small Ruminant Res. 65, 266-273.

Kim, K.-S., Min, M.-S., An, J.-H. & Lee, H., 2004. Cross-species amplification of bovidae microsatellites and low diversity of the endangered Korean Goral. J. Hered. 95, 521-525.

Kosgey, I.S. & Okeyo, A.M., 2007. Genetic improvement of small ruminants in low-input, smallholder production systems: Technical and infrastructural issues. Small Ruminant Res. 70, 76-88.

Kumar, D., Dixit, S.P., Sharma, R., Pandey, A.K., Sirohi, G., Patel, A.K., Aggarwal, R.A.K., Verma, N.K., Gour, D.S. & Ahlawat, S.P.S., 2005. Population structure, genetic variation and management of Marwari goats. Small Ruminant Res. 59, 41-48.

Li, J.Y., Chen, H., Lan, X.Y., Kong, X.J. & Min, L.J., 2008. Genetic diversity of five Chinese goat breeds assessed by microsatellite markers. Czech J. Anim. Sci. 53, 315-319.

Lopes, F.B., Borjas, A.d.l.R., da Silva, M.C., Facó, O., Lôbo, R.N., Fiorvanti, M.C.S. & McManus, C., 2012. Breeding goals and selection criteria for intensive and semi-intensive dairy goat system in Brazil. Small Ruminant Res. 106, 110-117.

Maddox, J.F. & Cockett, N.E., 2007. An update on sheep and goat linkage maps and other genomic resources. Small Ruminant Res. 70, 4-20.

Maiwashe, A., Nephawe, K.A., Van der Westhuizen, R.R., Mostert, B.E. & Theron, H.E., 2006. Rate of inbreeding and effective population size in four major South African dairy cattle breeds. S. Afr. J. Anim. Sci. 36, 50-57.

Martínez, A.M., Acosta, J., Vega-Pla, J.L. & Delgado, J.V., 2006. Analysis of the genetic structure of the Canary goat populations using microsatellites. Livest. Sci. 102, 140-145.

Montaldo, H.H. & Manfredi, E., 2002. Organisation of selection programmes for dairy goats. Proc. 7th Wrld. Congr. Genet. Appl. Livest. Prod. Vol. 29, Montpellier, France. pp. 187-194.

Morin, P.A., Luikart, G., Wayne, R.K. & the SNP workshop group. 2004. SNPs in ecology, evolution and conservation. Trends Ecol. Evol. 19, 208-216.

Morris, C.A., Wheeler, M., Hosking, B.C., Watson, T.G., Hurford, A.P., Foote, B.J. & Foote, J.F., 1997. Genetic parameters for milk yield and faecal nematode egg count in Saanen goats. New Zealand J. Agric. Res. 40, 523-528.

Mucha, S. & Windig, J.J., 2009. Effects of incomplete pedigree on genetic management of the Dutch Landrace goat. J. Anim. Breed. Genet. 126, 250-256.

Muller, C.J.C., 2005. Genetic parameter estimation and breeding plans for the South African dairy goat herd. Ph.D. Thesis. Department of Animal Sciences,, Faculty of Agricultural and Forestry Sciences, University of Stellenbosch. Stellenbosch.

Naderi, S., Rezaei, H.-R., Pompanon, F., Blum, M.G.B., Negrini, R., Naghash, H.-R., Balkiz, Ö., Mashkour, M., Gaggiotti, O.E., Ajmone-Marsan, P., Kence, A., Vigne, J.-D. & Taberlet, P., 2008. The goat domestication process inferred from large-scale mitochondrial DNA analysis of wild and domestic individuals. Proc. Natl. Acad. Sci. U. S. A. 105, 17659-17664.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89, 583-590.

Nei, M., 1987. Molecular evolutionary genetics. Columbia University Press, New York. 164-165.

Olivier, J.J., Cloete, S.W.P., Schoeman, S.J. & Muller, C.J.C., 2005. Performance testing and recording in meat and dairy goats. Small Ruminant Res. 60, 83-93.

Park, S.D.E., 2001. Trypanotolerance in West African cattle and the population genetic effects of selection [Ph.D. thesis] University of Dublin.

Peacock, C., 2005. Goats—A pathway out of poverty. Small Ruminant Res. 60, 179-186.

Pieters, A., Van Marle-Köster, E., Visser, C. & Kotze, A., 2009. South African developed meat type goats: a forgotten animal genetic resource? Anim. Genet. Res. 44, 33-43.

Pritchard, J.K., Stephens, M. & Donnelly, P., 2000. Inference of population structure using multilocus genotye data. Genetics. 155, 945-959.

Qi, Y., Luo, J., Han, X., Zhu, Y., Chen, C., Liu, J. & Sheng, H., 2009. Genetic diversity and relationships of 10 Chinese goat breeds in the Middle and Western China. Small Ruminant Res. 82, 88-93.

South African Milch Goat Breeders' Society, 2013. Breeds. http://www.milkgoats.co.za/milkgoat_society/index.php?p=59 accessed on 24 January 2013.

Sahlu, T. & Goetsch, A.L., 2005. A foresight on goat research. Small Ruminant Res. 60, 7-12.

Saitbekova, N., Gaillard, C., Obexer-Ruff, G. & Dolf, G., 1999. Genetic diversity in Swiss goat breeds based on microsatellite analysis. Anim. Genet. 30, 36-41.

Shrestha, J.N.B., 2005. Conserving domestic animal diversity among composite populations. Small Ruminant Res. 56, 3-20.

Stachowicz, K., Sargolzaei, M., Miglior, F. & Schenkel, F.S., 2011. Rates of inbreeding and genetic diversity in Canadian Holstein and Jersey cattle. J. Dairy Sci. 94, 5160-5175.

Taberlet, P., Coissac, E., Pansu, J. & Pompanon, F., 2011. Conservation genetics of cattle, sheep, and goats. C. R. Biol. 334, 247-254.

Tamaki, K. & Jeffreys, A.J., 2005. Human tandem repeat sequences in forensic DNA typing. Leg. Med. 7, 244-250.

Tolone, M., Mastrangelo, S., Rosa, A.J.M. & Portolano, B., 2012. Genetic diversity and population structure of Sicilian sheep breeds using microsatellite markers. Small Ruminant Res. 102, 18-25.

Toro, M.A., Fernández, J. & Caballero, A., 2009. Molecular characterization of breeds and its use in conservation. Livest. Sci. 120, 174-195.

Torres-Vázquez, J.A., Valencia-Posadas, M., Castillo-Juárez, H. & Montaldo, H.H., 2009. Genetic and phenotypic parameters of milk yield, milk composition and age at first kidding in Saanen goats from Mexico. Livest. Sci. 126, 147-153.

Traoré, A., Álvarez, I., Tamboura, H.H., Fernández, I., Kaboré, A., Royo, L.J., Gutiérrez, J.P., Sangaré, M., Ouédraogo-Sanou, G., Toguyeni, A., Sawadogo, L. & Goyache, F., 2009. Genetic characterisation of Burkina Faso goats using microsatellite polymorphism. Livest. Sci. 123, 322-328.

Villanueva, B., Sawalha, R.M., Roughsedge, T., Rius-Vilarrasa, E. & Woolliams, J.A., 2010. Development of a genetic indicator of biodiversity for farm animals. Livest. Sci. 129, 200-207.

Visser, C., 2010. A molecular approach to genetic improvement of South African Angora goats. PhD Thesis. Faculty of Natural and Agricultural Sciences, University of Pretoria. Pretoria.

Visser, C., Crooijmans, R.P.M.A. & Van Marle-Köster, E., 2010. A genetic linkage map for the South African Angora goat. Small Ruminant Res. 93, 171-179.

Visser, C., Van Marle-Köster, E., Bovenhuis, H. & Crooijmans, R.P.M.A., 2011a. QTL for mohair traits in South African Angora goats. Small Ruminant Res. 100, 8-14.

Visser, C., Van Marle-Köster, E. & Friedrich, H., 2011b. Parentage verification of South African Angora goats, using microsatellites markers. S. Afr. J. Anim. Sci. 41, 250-255.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Anim. Feed Sci. Tech. 147, 116-139.

Weir, B.S. & Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution. 38, 1358-1370.

Zane, L., Bargelloni, L. & Patarnello, T., 2002. Strategies for microsatellite isolation: a review. Mol. Ecol. 11, 1-16.

Addendum A

Survey Questionnaire

(Office Use) Record number:

Genetic Study: Dairy Goat Diversity

Information Sheet and Survey

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Departement of Animal and Wildlife Sciences
Faculty of Natural and Agricultural Sciences

Confidentiality Policy

All information provided in this document will be treated as **strictly confidential**.

NB: Guide to completing the survey

1. This form can be completed on the computer, or printed out and filled in.
2. Please use an "X" where applicable
3. The "Individual Animal Information" section pertains ONLY to the animals that will be sampled.
4. Should you have any queries when completing the survey, please feel free to contact me by email, or on 072 xxx xxxx (office hours).
5. You can submit the form via email to u2xxxxxxx@tuks.co.za, or fax to 012 xxx xxxx, for ATTENTION: Xxxxx Xxxxx.

Sections in this survey:

1. General Farm Information
2. Herd Management
3. Individual Animal Information

1. General Farm Information

Name: _____ Area: _____

Owner: _____ Contact number: _____

Years since herd establishment: _____

Products produced:
- Fresh Milk ☐
- Hard Cheese ☐
- Soft Cheese ☐
- Yogurt ☐
- Other (please specify) _____

Do you market products:
- Directly to the public (e.g. Farmer's markets) ☐
- To industry ☐

1

(Office Use) Record number:

2. Herd Management

Breeds in herd: British Alpine (BA.) ☐
Saanen (Sa.) ☐
Toggenburg (Tb.) ☐
Other (O.) (please specify) _____

Number of animals:

Bucks	BA. _____	Sa. _____	Tb. _____	O. _____
Does in milk	BA. _____	Sa. _____	Tb. _____	O. _____
Replacement does	BA. _____	Sa. _____	Tb. _____	O. _____

Do you make you use of: Pure breeding ☐ Crossbreeding ☐ Both ☐

Do you use selection indices, breeding values or other genetic information to select replacement stock? Yes ☐ No ☐

If **Yes**, please specify which of the following you use:

Selection index ☐
Breeding values ☐
Other (please specify): _____

When sourcing replacement stock, which of the following avenues do you use?

Bucks: Breed own ☐ Local co-breeder ☐ Import ☐

Does: Breed own ☐ Local co-breeder ☐ Import ☐

Do you make use of: Artificial insemination ☐ Natural service ☐ Both ☐

Do you make young bucks and/or does available for purchase? Yes ☐ No ☐

Do you keep: Pedigree records Yes ☐ No ☐

Production records Yes ☐ No ☐

Do you participate in a national recording scheme? Yes ☐ No ☐

Would you be interested in a pedigree analysis for your own herd? Yes ☐ No ☐

Would you be willing to make your records available for a national pedigree analysis? Yes ☐ No ☐

(Office Use) Record number:

3. Individual Animal Information

Animal ID: ______________________ **Lab Number:**

Breed: ______________ Sex: M ☐ F ☐ Age: ________

Sire: ______________________ Dam: ________

Grandsire: ______________________

Animal ID: ______________________ **Lab Number:**

Breed: ______________ Sex: M ☐ F ☐ Age: ________

Sire: ______________________ Dam: ________

Grandsire: ______________________

Animal ID: ______________________ **Lab Number:**

Breed: ______________ Sex: M ☐ F ☐ Age: ________

Sire: ______________________ Dam: ________

Grandsire: ______________________

Animal ID: ______________________ **Lab Number:**

Breed: ______________ Sex: M ☐ F ☐ Age: ________

Sire: ______________________ Dam: ________

Grandsire: ______________________

Animal ID: ______________________ **Lab Number:**

Breed: ______________ Sex: M ☐ F ☐ Age: ________

Sire: ______________________ Dam: ________

Grandsire: ______________________

Addendum B

Amplification Success Rates

Locus	Animals genotyped	Number of genotypes assigned	Amplification success rate
BM1258	240	240	100.0%
BM1329	240	239	99.6%
BM1818	240	240	100.0%
BM7160	240	239	99.6%
CSRD247	240	239	99.6%
HSC	240	237	98.8%
ILSTS005	240	240	100.0%
ILSTS011	240	237	98.8%
ILSTS087	240	240	100.0%
INRA23	240	236	98.3%
INRA40	240	235	97.9%
INRA63	240	239	99.6%
INRA132	240	239	99.6%
INRABERN172	240	239	99.6%
INRABERN185	240	239	99.6%
INRABERN192	240	240	100.0%
MAF65	240	239	99.6%
MAF209	240	240	100.0%
MCM527	240	240	100.0%
OarFCB20	240	240	100.0%
OarFCB48	240	239	99.6%
OarFCB128	240	238	99.2%
SRCRSP5	240	240	100.0%
SRCRSP8	240	239	99.6%
SRCRSP9	240	239	99.6%
Average			**99.5%**

Addendum C

Table of Allelic Frequencies

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
BM1258	1	99	0.0077	0.2255	0.0169	0.0563	
BM1258	2	101	0.2692	0.1176	0.2288	0.2271	
BM1258	3	103	0.2923	0.2941	0.2119	0.2729	
BM1258	4	105	0.1923	0.0686	0.1271	0.15	
BM1258	5	107	0.0423	0.0588	0.1356	0.0688	
BM1258	6	109	0.0808	0.0196	0.1525	0.0854	
BM1258	7	111	0.0269	0.0098	0.0424	0.0271	
BM1258	8	113	0.0385	0	0.0169	0.025	
BM1258	9	115	0.0038	0.0196	0.0254	0.0125	
BM1258	10	119	0.0077	0.0196	0	0.0083	
BM1258	11	121	0.0346	0.1667	0.0424	0.0646	
BM1258	12	123	0.0038	0	0	0.0021	Saanen
BM1258	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
BM1329	1	170	0.155	0.0294	0.0424	0.1004	
BM1329	2	172	0.2907	0.3137	0.1864	0.2699	
BM1329	3	174	0	0.0784	0.0339	0.0251	
BM1329	4	176	0.0039	0	0.0169	0.0063	
BM1329	5	178	0.3798	0.4804	0.6864	0.477	
BM1329	6	180	0.1667	0.0882	0.0169	0.113	
BM1329	7	182	0.0039	0.0098	0.0169	0.0084	
BM1329	#	samples:	129	51	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
BM1818	1	254	0.0385	0.0294	0.0763	0.0458	
BM1818	2	256	0.3346	0.4412	0.1949	0.3229	
BM1818	3	258	0.0923	0.1275	0.2881	0.1479	
BM1818	4	260	0.2462	0.0882	0.2288	0.2083	
BM1818	5	262	0.0885	0.2059	0.1102	0.1187	
BM1818	6	264	0.1423	0.1078	0.0763	0.1187	
BM1818	7	266	0.0577	0	0.0254	0.0375	
BM1818	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
BM7160	1	167	0.0271	0.2745	0.0254	0.0795	
BM7160	2	169	0.0543	0.0098	0.0508	0.0439	
BM7160	3	173	0.0116	0	0.0847	0.0272	
BM7160	4	175	0.3062	0.2157	0.5763	0.3536	
BM7160	5	177	0.2829	0.2843	0.1949	0.2615	
BM7160	6	179	0.0233	0.0294	0	0.0188	
BM7160	7	181	0.2868	0.1863	0.0678	0.2113	
BM7160	8	183	0.0078	0	0	0.0042	Saanen
BM7160	#	samples:	129	51	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
CSRD247	1	219	0.0115	0	0.0339	0.0146	
CSRD247	2	231	0.2077	0.31	0.3983	0.2762	
CSRD247	3	233	0.5538	0.52	0.4407	0.5188	
CSRD247	4	235	0.15	0.04	0.0169	0.0941	
CSRD247	5	239	0.0423	0.01	0.0593	0.0397	
CSRD247	6	241	0.0038	0.09	0.0254	0.0272	
CSRD247	7	243	0.0308	0.02	0.0254	0.0272	
CSRD247	8	245	0	0.01	0	0.0021	Toggenburg
CSRD247	#	samples:	130	50	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
HSC	1	269	0	0.0588	0.1724	0.0549	
HSC	2	271	0.0703	0.1569	0	0.0717	
HSC	3	273	0.1836	0.2255	0.069	0.1646	
HSC	4	275	0.1133	0.0294	0	0.0675	
HSC	5	277	0.0078	0	0	0.0042	Saanen
HSC	6	281	0.0859	0.3039	0.1207	0.1414	
HSC	7	283	0.3047	0.1667	0.5431	0.3333	
HSC	8	285	0.1016	0.0196	0.0345	0.0675	
HSC	9	287	0.1094	0.0098	0.0086	0.0633	
HSC	10	289	0.0234	0.0098	0.0431	0.0253	
HSC	11	291	0	0.0098	0.0086	0.0042	
HSC	12	299	0	0.0098	0	0.0021	Toggenburg
HSC	#	samples:	128	51	58	237	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
ILSTS005	1	177	0.0154	0.0294	0.1102	0.0417	
ILSTS005	2	179	0	0	0.0085	0.0021	British Alpine
ILSTS005	3	181	0.7962	0.598	0.4915	0.6792	
ILSTS005	4	183	0.1731	0.3725	0.3898	0.2687	
ILSTS005	5	189	0.0154	0	0	0.0083	Saanen
ILSTS005	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
ILSTS011	1	267	0	0.07	0.0254	0.0211	
ILSTS011	2	269	0.1016	0.04	0.1102	0.0907	
ILSTS011	3	271	0.0781	0.04	0.0424	0.0612	
ILSTS011	4	273	0	0	0.0508	0.0127	British Alpine
ILSTS011	5	275	0	0	0.0339	0.0084	British Alpine
ILSTS011	6	277	0.3711	0.34	0.3983	0.3713	
ILSTS011	7	279	0.3203	0.39	0.2966	0.3291	
ILSTS011	8	281	0.1055	0.02	0.0085	0.0633	
ILSTS011	9	283	0.0234	0.1	0.0339	0.0422	
ILSTS011	#	samples:	128	50	59	237	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
ILSTS087	1	139	0	0	0.0593	0.0146	British Alpine
ILSTS087	2	141	0.0769	0.2059	0.0169	0.0896	
ILSTS087	3	143	0.0192	0.0588	0.0254	0.0292	
ILSTS087	4	145	0.1462	0.4216	0.4831	0.2875	
ILSTS087	5	147	0	0	0.0085	0.0021	British Alpine
ILSTS087	6	149	0.0577	0.1078	0.0339	0.0625	
ILSTS087	7	151	0.1923	0.0686	0.178	0.1625	
ILSTS087	8	153	0.5	0.0882	0.1441	0.325	
ILSTS087	9	155	0.0038	0	0	0.0021	Saanen
ILSTS087	10	157	0.0038	0.049	0.0508	0.025	
ILSTS087	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRA23	1	197	0	0	0.0088	0.0021	British Alpine
INRA23	2	199	0.031	0.2	0.1491	0.0953	
INRA23	3	201	0.1202	0.04	0.0439	0.0847	
INRA23	4	203	0.0078	0.15	0	0.036	
INRA23	5	205	0.0039	0	0.0351	0.0106	
INRA23	6	207	0	0.01	0.0088	0.0042	
INRA23	7	211	0.0349	0.02	0.0351	0.0318	
INRA23	8	213	0.7364	0.58	0.7193	0.6992	
INRA23	9	215	0.0659	0	0	0.036	Saanen
INRA23	#	samples:	129	50	57	236	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRA40	1	222	0.1151	0.01	0.0424	0.0745	
INRA40	2	224	0.004	0.08	0.0085	0.0213	
INRA40	3	230	0.0397	0.02	0	0.0255	
INRA40	4	232	0.0317	0.07	0.0169	0.0362	
INRA40	5	236	0.4008	0.06	0.178	0.2723	
INRA40	6	238	0.0357	0.02	0.0169	0.0277	
INRA40	7	240	0.0437	0.13	0.1695	0.0936	
INRA40	8	242	0.0278	0.06	0.0763	0.0468	
INRA40	9	244	0.1151	0.27	0.2627	0.1851	
INRA40	10	246	0.123	0.27	0.2119	0.1766	
INRA40	11	248	0.0635	0.01	0.0169	0.0404	
INRA40	#	samples:	126	50	59	235	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRA63	1	161	0.1163	0.0196	0.0508	0.0795	
INRA63	2	163	0.1279	0.1765	0.1695	0.1485	
INRA63	3	165	0.5039	0.4412	0.3644	0.4561	
INRA63	4	167	0.2287	0.3627	0.4068	0.3013	
INRA63	5	169	0.0233	0	0.0085	0.0146	
INRA63	#	samples:	129	51	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRA132	1	131	0.1077	0.01	0.0424	0.0711	
INRA132	2	137	0.0077	0.02	0	0.0084	
INRA132	3	139	0.4885	0.6	0.4661	0.5063	
INRA132	4	141	0.3962	0.28	0.4322	0.3808	
INRA132	5	143	0	0.02	0.0254	0.0105	
INRA132	6	151	0	0.01	0.0169	0.0063	
INRA132	7	155	0	0.06	0.0169	0.0167	
INRA132	#	samples:	130	50	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRABERN172	1	233	0.0154	0.03	0.0424	0.0251	
INRABERN172	2	237	0.0423	0.04	0.1356	0.0649	
INRABERN172	3	239	0.3538	0.2	0.2627	0.2992	
INRABERN172	4	241	0.0192	0.14	0.0085	0.0418	
INRABERN172	5	243	0.1038	0.06	0.1102	0.0962	
INRABERN172	6	245	0.4308	0.52	0.2458	0.4038	
INRABERN172	7	247	0.0346	0.01	0.178	0.0649	
INRABERN172	8	251	0	0	0.0169	0.0042	British Alpine
INRABERN172	#	samples:	130	50	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRABERN185	1	265	0.6731	0.7255	0.7931	0.7134	
INRABERN185	2	267	0	0.0196	0	0.0042	Toggenburg
INRABERN185	3	277	0	0	0.0172	0.0042	British Alpine
INRABERN185	4	281	0.1923	0.2549	0.181	0.2029	
INRABERN185	5	287	0.1346	0	0.0086	0.0753	
INRABERN185	#	samples:	130	51	58	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
INRABERN192	1	176	0.0077	0	0	0.0042	Saanen
INRABERN192	2	182	0.4154	0.0294	0.1186	0.2604	
INRABERN192	3	184	0	0	0.0085	0.0021	British Alpine
INRABERN192	4	186	0.5115	0.6667	0.5678	0.5583	
INRABERN192	5	188	0.0038	0.0294	0.1695	0.05	
INRABERN192	6	190	0.0231	0.0196	0.0339	0.025	
INRABERN192	7	194	0.0346	0.2451	0.1017	0.0958	
INRABERN192	8	196	0.0038	0	0	0.0021	Saanen
INRABERN192	9	198	0	0.0098	0	0.0021	Toggenburg
INRABERN192	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
MAF65	1	118	0.0271	0.0392	0.0763	0.0418	
MAF65	2	120	0.1008	0.0098	0.2034	0.1067	
MAF65	3	122	0.0659	0.0196	0.0085	0.0418	
MAF65	4	124	0.0504	0.1373	0.0424	0.0669	
MAF65	5	126	0.0039	0.0392	0.0339	0.0188	
MAF65	6	128	0.1279	0.0686	0.0254	0.09	
MAF65	7	132	0.3178	0.1961	0.4068	0.3138	
MAF65	8	134	0.0891	0.4314	0.0678	0.1569	
MAF65	9	136	0.2171	0.0588	0.1356	0.1632	
MAF65	#	samples:	129	51	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
MAF209	1	105	0.0385	0.049	0.1949	0.0792	
MAF209	2	107	0.7654	0.8627	0.7373	0.7792	
MAF209	3	109	0.1962	0.0882	0.0678	0.1417	
MAF209	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
MCM527	1	155	0.4731	0.4706	0.6441	0.5146	
MCM527	2	157	0	0.1176	0.0593	0.0396	
MCM527	3	165	0.0808	0.0686	0.0424	0.0688	
MCM527	4	167	0.0154	0.2745	0.1102	0.0938	
MCM527	5	169	0.0192	0.0098	0	0.0125	
MCM527	6	171	0.4115	0.049	0.1441	0.2687	
MCM527	7	173	0	0.0098	0	0.0021	Toggenburg
MCM527	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
OarFCB20	1	91	0.0077	0	0.0085	0.0063	
OarFCB20	2	93	0.2308	0.0686	0.1356	0.1729	
OarFCB20	3	95	0.3308	0.8725	0.5254	0.4938	
OarFCB20	4	97	0.2192	0.049	0.2034	0.1792	
OarFCB20	5	99	0.0885	0	0.0254	0.0542	
OarFCB20	6	101	0.1231	0.0098	0.1017	0.0938	
OarFCB20	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
OarFCB48	1	156	0.1423	0.02	0.0254	0.0879	
OarFCB48	2	160	0.0385	0.03	0.0254	0.0335	
OarFCB48	3	164	0.1423	0.43	0.2627	0.2322	
OarFCB48	4	166	0.1115	0.15	0.2288	0.1485	
OarFCB48	5	168	0.3692	0.23	0.3644	0.3389	
OarFCB48	6	170	0.1962	0.14	0.0763	0.1548	
OarFCB48	7	172	0	0	0.0169	0.0042	British Alpine
OarFCB48	#	samples:	130	50	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
OarFCB128	1	98	0	0	0.0169	0.0042	British Alpine
OarFCB128	2	100	0.6562	0.9412	0.7373	0.7374	
OarFCB128	3	102	0.3359	0.0588	0.1949	0.2416	
OarFCB128	4	104	0.0078	0	0.0508	0.0168	
OarFCB128	#	samples:	128	51	59	238	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
SRCRSP5	1	162	0.0346	0.0784	0.0254	0.0417	
SRCRSP5	2	164	0.0462	0.049	0.0339	0.0437	
SRCRSP5	3	168	0.1231	0.1765	0.1271	0.1354	
SRCRSP5	4	170	0.0423	0.2451	0.0763	0.0938	
SRCRSP5	5	172	0.7385	0.3725	0.7288	0.6583	
SRCRSP5	6	176	0.0038	0	0	0.0021	Saanen
SRCRSP5	7	178	0.0077	0.0784	0	0.0208	
SRCRSP5	8	180	0	0	0.0085	0.0021	British Alpine
SRCRSP5	9	182	0.0038	0	0	0.0021	Saanen
SRCRSP5	#	samples:	130	51	59	240	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
SRCRSP8	1	217	0.0346	0.22	0.0085	0.0669	
SRCRSP8	2	219	0	0.01	0	0.0021	Toggenburg
SRCRSP8	3	227	0.0269	0.17	0.2373	0.1088	
SRCRSP8	4	229	0.2231	0.04	0.0678	0.1464	
SRCRSP8	5	231	0.1885	0.13	0.1102	0.1569	
SRCRSP8	6	233	0.0192	0	0.0169	0.0146	
SRCRSP8	7	237	0.1192	0.11	0.322	0.1674	
SRCRSP8	8	239	0	0.04	0.0085	0.0105	
SRCRSP8	9	241	0	0	0.0085	0.0021	British Alpine
SRCRSP8	10	243	0.0115	0.01	0.0085	0.0105	
SRCRSP8	11	247	0.3769	0.27	0.2034	0.3117	
SRCRSP8	12	249	0	0	0.0085	0.0021	British Alpine
SRCRSP8	#	samples:	130	50	59	239	

Locus	Allele#	Size	Saanen	Toggenburg	British Alpine	Overall	Private?
SRCRSP9	1	120	0.0038	0.049	0.0086	0.0146	
SRCRSP9	2	122	0.0231	0	0	0.0126	Saanen
SRCRSP9	3	124	0.0038	0.0392	0.0603	0.0251	
SRCRSP9	4	126	0.2115	0.598	0.4052	0.341	
SRCRSP9	5	128	0.3077	0.1275	0.181	0.2385	
SRCRSP9	6	130	0.0038	0	0	0.0021	Saanen
SRCRSP9	7	134	0.1962	0.1078	0.1552	0.1674	
SRCRSP9	8	136	0.0462	0.0098	0.1207	0.0565	
SRCRSP9	9	138	0.0038	0	0	0.0021	Saanen
SRCRSP9	10	140	0.0077	0.049	0.069	0.0314	
SRCRSP9	11	142	0.1923	0.0196	0	0.1088	
SRCRSP9	#	samples:	130	51	58	239	

Addendum D

Summary Statistics of the Saanen, Toggenburg and British Alpine Breeds

Summary of the expected (H_E) and observed (H_O) heterozygosity, polymorphic information content (PIC), Hardy Weinberg equilibrium (HWE) and Wright's *F*-statistics for the Saanen breed

Locus	**H_O**	**H_E**	**PIC**	**HWE**	***F_{IT} (F)***	***F_{ST} (Θ)***	***F_{IS} (f)***
BM1258	0.677	0.796	0.764	**0.00873 ± 0.00007**	0.160	0.065	0.101
BM1329	0.729	0.722	0.670	0.96955 ± 0.00019	-0.006	0.018	-0.024
BM1818	0.800	0.789	0.757	0.97654 ± 0.00015	0.001	0.085	-0.093
BM7160	0.674	0.742	0.694	**0.00231 ± 0.00004**	0.101	0.058	0.045
CSRD247	0.623	0.627	0.580	0.27633 ± 0.00041	0.010	0.018	-0.008
HSC	0.797	0.829	0.805	0.25038 ± 0.00039	0.044	0.036	0.009
ILSTS005	0.346	0.337	0.297	0.70515 ± 0.0005	-0.019	0.049	-0.071
ILSTS011	0.766	0.734	0.689	0.87705 ± 0.00031	-0.037	0.030	-0.069
ILSTS087	0.677	0.685	0.645	0.19619 ± 0.00038	0.023	0.071	-0.051
INRA132	0.546	0.595	0.509	**0.02683 ± 0.0002**	0.083	0.001	0.082
INRA23	0.426	0.438	0.414	0.84912 ± 0.00026	0.036	0.055	-0.019
INRA40	0.619	0.790	0.768	**0 ± 0**	0.224	0.052	0.182
INRA63	0.612	0.666	0.618	**0.0122 ± 0.00011**	0.094	0.085	0.010
INRABERN172	0.592	0.677	0.619	0.11217 ± 0.00029	0.131	0.035	0.100
INRABERN185	0.523	0.494	0.441	0.07728 ± 0.00029	-0.051	0.050	-0.106
INRABERN192	0.523	0.566	0.472	0.3426 ± 0.00033	0.088	0.071	0.017
MAF209	0.362	0.376	0.327	0.25925 ± 0.00044	0.046	0.050	-0.005
MAF65	0.837	0.813	0.786	0.48483 ± 0.00044	-0.018	0.068	-0.092
MCM527	0.669	0.602	0.518	0.16641 ± 0.00035	-0.101	0.061	-0.172
OarFCB128	0.438	0.458	0.359	0.66586 ± 0.00046	0.050	0.028	0.022
OarFCB20	0.792	0.769	0.729	0.77423 ± 0.00034	-0.028	0.012	-0.040
OarFCB48	0.777	0.774	0.739	0.79465 ± 0.00036	0.007	0.063	-0.060
SRCRSP5	0.423	0.436	0.412	0.26348 ± 0.00033	0.032	0.013	0.019
SRCRSP8	0.754	0.759	0.721	0.67661 ± 0.00044	0.016	0.055	-0.041
SRCRSP9	0.669	0.785	0.749	0.11818 ± 0.00022	0.162	0.094	0.075
Average	0.626	0.650	0.603		0.046 ± 0.017	0.05 ± 0.005	-0.005 ± 0.017

Summary of the expected (H_E) and observed (H_O) heterozygosity, polymorphic information content (PIC), Hardy Weinberg equilibrium (HWE) and Wright's *F*-statistics for the Toggenburg breed

Locus	**H_O**	**H_E**	**PIC**	**HWE**	***F_{IT} (F)***	***F_{ST} (Θ)***	***F_{IS} (f)***
BM1258	0.784	0.820	0.787	0.21657 ± 0.0003	0.057	0.081	-0.027
BM1329	0.686	0.662	0.601	0.99764 ± 0.00005	-0.015	0.118	-0.152
BM1818	0.784	0.734	0.692	0.78515 ± 0.00034	-0.055	0.079	-0.146
BM7160	0.725	0.769	0.720	0.16582 ± 0.00038	0.054	-0.020	0.073
CSRD247	0.660	0.629	0.564	0.98022 ± 0.00012	-0.042	0.040	-0.085
HSC	0.804	0.807	0.772	0.29218 ± 0.00027	0.005	0.008	-0.002
ILSTS005	0.490	0.508	0.403	0.44729 ± 0.00047	0.036	0.009	0.028
ILSTS011	0.900	0.721	0.669	**0.00066 ± 0.00002**	-0.251	0.002	-0.254
ILSTS087	0.765	0.757	0.721	0.21946 ± 0.00036	-0.001	0.053	-0.057
INRA132	0.460	0.563	0.497	0.1598 ± 0.00031	0.202	0.123	0.090
INRA23	0.600	0.605	0.554	0.27328 ± 0.00029	0.010	0.010	0.000
INRA40	0.820	0.826	0.796	**0.00006 ± 0.00001**	0.018	0.061	-0.046
INRA63	0.608	0.649	0.570	0.6009 ± 0.0005	0.075	0.074	0.002
INRABERN172	0.700	0.671	0.626	0.13829 ± 0.00031	-0.035	0.052	-0.092
INRABERN185	0.431	0.412	0.340	**0.00837 ± 0.00008**	-0.036	0.060	-0.102
INRABERN192	0.529	0.498	0.438	0.98446 ± 0.00012	-0.061	0.012	-0.075
MAF209	0.275	0.248	0.230	**1 ± 0**	-0.089	0.101	-0.212
MAF65	0.784	0.752	0.716	**0.01518 ± 0.00012**	-0.029	0.078	-0.116
MCM527	0.667	0.689	0.636	0.57682 ± 0.00064	0.040	0.044	-0.004
OarFCB128	0.118	0.112	0.105	**1 ± 0**	-0.050	0.014	-0.065
OarFCB20	0.235	0.234	0.220	0.57755 ± 0.00041	-0.002	0.027	-0.030
OarFCB48	0.860	0.726	0.678	0.57206 ± 0.00041	-0.179	0.038	-0.225
SRCRSP5	0.824	0.763	0.719	0.61175 ± 0.0004	-0.067	0.074	-0.152
SRCRSP8	0.720	0.826	0.793	0.22319 ± 0.00030	0.142	0.088	0.060
SRCRSP9	0.608	0.614	0.582	0.56478 ± 0.00047	0.022	0.070	-0.052
Average	0.634	0.624	0.577		-0.006 ± 0.020	0.053 ± 0.008	-0.063 ± 0.020

Summary of the expected (H_E) and observed (H_O) heterozygosity, polymorphic information content (PIC), Hardy Weinberg equilibrium (HWE) and Wright's *F*-statistics for the British Alpine breed

Locus	**H_O**	**H_E**	**PIC**	**HWE**	***F_{IT} (F)***	***F_{ST} (Θ)***	***F_{IS} (f)***
BM1258	0.763	0.847	0.821	**0.00998 ± 0.00014**	0.113	0.088	0.027
BM1329	0.525	0.494	0.454	0.48968 ± 0.00042	-0.040	0.136	-0.204
BM1818	0.881	0.809	0.774	0.22899 ± 0.00035	-0.086	0.021	-0.110
BM7160	0.559	0.620	0.578	0.09948 ± 0.00031	0.108	0.064	0.047
CSRD247	0.695	0.646	0.575	0.16934 ± 0.00035	-0.070	0.031	-0.105
HSC	0.655	0.658	0.620	0.66082 ± 0.00034	0.004	-0.007	0.011
ILSTS005	0.644	0.599	0.511	0.13874 ± 0.00032	-0.073	0.012	-0.086
ILSTS011	0.712	0.740	0.696	**0.00006 ± 0.00001**	0.041	0.014	0.027
ILSTS087	0.797	0.712	0.675	0.75546 ± 0.0005	-0.116	0.022	-0.141
INRA132	0.593	0.598	0.509	0.21888 ± 0.00033	0.014	0.040	-0.027
INRA23	0.333	0.460	0.428	**0.02354 ± 0.00016**	0.286	0.081	0.223
INRA40	0.831	0.824	0.792	**0.00008 ± 0.00001**	0.002	0.060	-0.062
INRA63	0.593	0.676	0.608	0.05094 ± 0.0002	0.139	0.111	0.031
INRABERN172	0.746	0.813	0.779	0.68044 ± 0.00046	0.090	0.042	0.050
INRABERN185	0.379	0.341	0.296	0.82616 ± 0.00042	-0.103	0.064	-0.179
INRABERN192	0.610	0.629	0.586	0.54706 ± 0.00051	0.035	0.034	0.001
MAF209	0.441	0.417	0.367	**1 ± 0**	-0.049	0.045	-0.098
MAF65	0.763	0.767	0.733	0.34274 ± 0.00054	0.013	0.043	-0.032
MCM527	0.475	0.552	0.514	0.06143 ± 0.00023	0.160	0.140	0.023
OarFCB128	0.424	0.419	0.371	**0.03279 ± 0.00018**	-0.013	-0.012	-0.001
OarFCB20	0.695	0.659	0.611	0.07491 ± 0.00029	-0.056	-0.005	-0.051
OarFCB48	0.763	0.745	0.695	0.69036 ± 0.00048	-0.018	0.037	-0.058
SRCRSP5	0.424	0.449	0.419	0.27939 ± 0.00038	0.078	0.146	-0.079
SRCRSP8	0.780	0.788	0.749	0.45101 ± 0.00033	0.021	0.069	-0.051
SRCRSP9	0.776	0.763	0.725	0.73862 ± 0.00036	-0.007	0.065	-0.077
Average	0.634	0.641	0.596		0.019 ± 0.017	0.052 ± 0.008	-0.035 ± 0.015

Addendum E

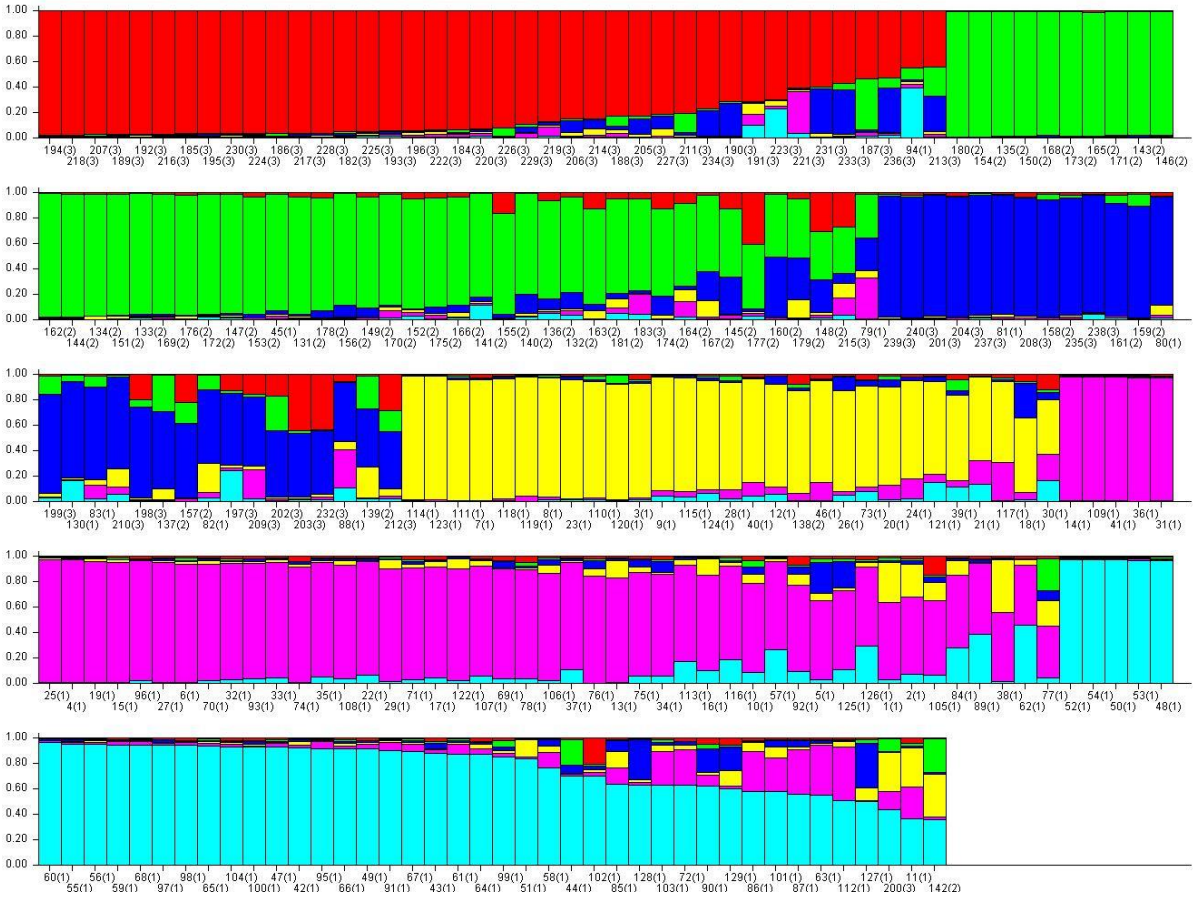
Wright's *F*-statistics

Wright's *F*-statistics for 25 microsatellite loci (F_{IT}, F_{ST} and F_{IS}) for each locus over all populations

Locus	F_{IT} *(F)*	F_{ST} *(Θ)*	F_{IS} *(f)*
BM1258	0.146	0.036	**0.115**
BM1329	0.034	0.058	-0.025
BM1818	-0.005	0.037	-0.044
BM7160	0.156	0.077	0.086
CSRD247	0.004	0.029	-0.026
HSC	0.099	0.076	0.024
ILSTS005	0.071	0.097	-0.029
ILSTS011	-0.056	0.009	-0.065
ILSTS087	0.113	0.135	-0.026
INRA23	0.121	0.044	0.080
INRA40	0.172	0.065	**0.114**
INRA63	0.108	0.022	0.088
INRA132	0.097	0.014	0.084
INRABERN172	0.120	0.043	0.080
INRABERN185	-0.044	0.022	-0.067
INRABERN192	0.144	0.110	0.038
MAF65	0.047	0.069	-0.024
MAF209	0.028	0.038	-0.010
MCM527	0.082	0.101	-0.021
OarFCB20	0.098	0.127	-0.034
OarFCB48	0.002	0.045	-0.045
OarFCB128	0.117	0.095	0.024
SRCRSP5	0.084	0.084	0.000
SRCRSP8	0.099	0.067	0.035
SRCRSP9	0.158	0.082	0.083
Average	0.083 ± 0.013	0.064 ± 0.007	0.020 ± 0.013

Addendum F

Structure Results



Expanded bar plot of K = 6, ordered according to *Q*, giving the animal lab numbers and where breeds are denoted by (1) Saanen, (2) Toggenburg and (3) British Alpine