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The impact of DNA parentage verification on EBV estimation and sire ranking in South African Angora goats

by

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ABSTRACT

South Africa is the world's largest mohair-producing country, contributing over 50% of the world's mohair, and therefore genetic improvement of Angora goats in South Africa is imperative in order to maintain this position in the market and the quality of the mohair clip. Pedigree integrity is vital for the success of any breeding programme. DNA parentage testing has become a useful tool in amending inaccuracies in on-farm records of various species. Previous studies have determined that errors in pedigree records may have a negative effect of up to 15% on genetic improvement in livestock. In the current study the extent of incorrect paternity records was quantified in 381 South African Angora goats using a panel of 12 microsatellite markers selected for parentage verification. 14.3% of the on-farm records were missing or incorrect. The microsatellite marker panel had a CPE of 99.6%, PIC of 0.700 and average H_E of 0.738. Estimated Breeding Values for fleece traits (fibre diameter and fleece weight) and body weights (birth weight, weaning weight, eight month weight, yearling weight and 16 month weight) were estimated for 21 sires using ASREML, firstly using the breeder's recorded pedigree and secondly using the DNA marker-verified pedigree. Sires were ranked according to EBVs for each trait. The sires ranked lowest in the breeder's records for fibre diameter, birth weight and weaning weight were moved to the top three ranks in the DNA verified pedigree. The ranking for fleece weight was not as severely affected. The significant change in sire ranking after DNA pedigree verification confirms the importance of pedigree integrity for selection accuracy in the South African Angora goat industry.

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For from Him and through Him and to Him are all things. To Him be glory forever.

-Romans 11:36

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This dissertation is dedicated to my mom, Lucille Garritsen, a woman of grace, strength and integrity, who I admire most.

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
BLAD	Bovine Leucocyte Adhesion Deficiency
BLUP	Best Linear Unbiased Prediction
CPE	Combined probability of exclusion
CVM	Complex Vertebral Conformation
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate (A, C, G, T)
DUMPs	Deficiency of Uridine Monophosphate Synthase
EBV	Estimated Breeding Value
EDTA	Ethylenediaminetetraacetic acid
FABI	Forestry and Agricultural Biotechnology Institute
FAO	Food and Agriculture Organisation
FD	Fibre diameter
F_{Null}	Null allele frequency
FW	Fleece weight
GADI	Grootfontein Agricultural Development Institute
GEBV	Genomic EBV
GxE	Genotype Environment Interaction
h^2	Heritability
H_E	Expected heterozogosity
H_O	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
ISAG	International Society of Animal Genetics
LOD	Natural logarithm of combined likelihood
mM	Millimolar
NDAFF	National Department of Agriculture, Fisheries and Forestry
Ne	Effective population size
ng/µL	Nanograms per microlitre
NSIS	National Small Stock Improvement Scheme
PCR	Polymerase chain reaction
P_E	Exclusion probability for sire alone
PIC	Polymorphic information content

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pmol/ µL	Picomol per microlitre
RAPD	Randomly Amplified Polymorphic DNA
REML	Restricted Maximum Likelihood
RFLP	Restriction Fragment Length Polymorphism
ROI	Return on Investment
SAS	Statistical Analysis System
SD	Standard Deviation
SI	Selection index
SNP	Single Nucleotide Polymorphism
UK	United Kingdom
USA	United States of America
UV	Ultra Violet
VNTR	Variable Number Tandem Repeat
W0	Birth weight
W12	Yearling weight
W16	Sixteen month weight
W4	Weaning weight (four month weight)
W8	Eight month weight

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CHAPTER 1: Introduction

1.1 The South African mohair industry and global market

Mohair is a luxury fibre produced exclusively by Angora goats for the manufacture of luxury clothing and upholstery (Di Tfiana & Sepe, 2008). It is admired for its unique natural qualities including lustre (giving garments a silky sheen), its dyeing capacity, durability, crease resistance and lightweightedness (www.mohair.co.za). It forms a niche market for the manufacture of specialized garments that fetch a high price (Phan & Wortmann, 2000).

Before the mid-1970s, Turkey and the USA dominated the global mohair market. Since then, South Africa has become the leading producer worldwide, contributing around 54% to the world's mohair production (http://www.mohair.co.za/index.php/mohair-south-africa). The global production has changed drastically with regard to volume produced and proportional contribution of different countries. Changes in production are largely dependent on prices of opposing markets (such as wool) and emerging fashion trends. Global production peaked between 1983 and 1990 (Figure 1.1) and has since significantly decreased by more than 70% (from 26 to 6.6 million kg in 2003) (van der Westhuysen, 2005).

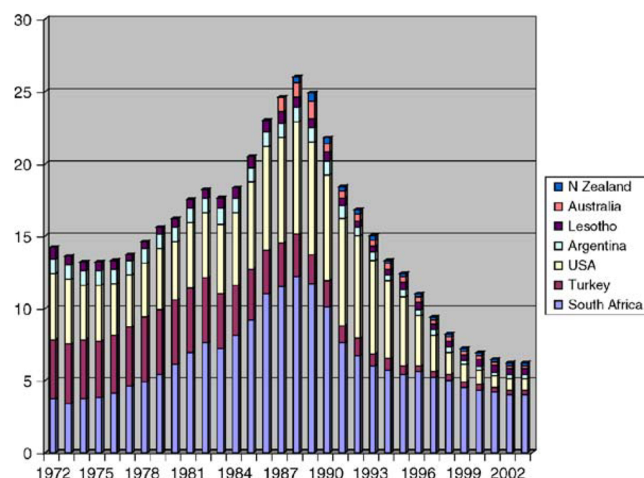

Figure 1.1 Mohair production in various countries (van der Westhuysen, 2005)

At present the global Angora population is approximately 2.6 million head, producing 6.6 million kg of mohair annually (van der Westhuysen, 2005). The South African Angora population is currently comprised of approximately 900 000 animals (Abstract of Agricultural Statistics, 2013, NDAFF, Republic of South Africa). The national mohair production was valued at R229 102 000 in August 2013, compared to wool, which grossed R2 087 639 000 and cotton which had a gross value of R211 932 000.

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Cotton is reported as the most predominantly manufactured natural fibre worldwide (FAO Apparel Fibre Consumption Survey, 2013), however the above statistics show that the monetary value held by mohair in South Africa exceeds that of cotton, demonstrating the important role played by the mohair industry in the South African agricultural (livestock) economy.

1.2 The South African Angora goat

Although there has been no documentation on the origin of the Angora goat, the earliest reported sightings were in the region of Ankara ("Angora") in Asia Minor, Turkey, around the year 1555 (Black, 1900; Pringle, 1989). It was believed that exceptional hair production was attributed exclusively to the country (its climate and environmental conditions),by pointing out that the Angora rabbit, cat and goat all produced "silky" hair (Black, 1900). The hair was seen as a protective shield for animals against the cold (Hayes, 1868; Black, 1900).

Initial attempts were made to export the animals to different areas in Europe, with very little success. In 1750 a shipment was sent out to France and in 1837 to England, soon after which all the animals died (Hayes, 1868), most probably owing to the climate differing so vastly from their native environment. The first export of Angora goats to the Cape of South Africa was made in 1838 (Black, 1900; Pringle, 1989). The animals thrived under South African conditions (possibly even better than their native habitat) and by 1857 a large flock of about 3000 Angora goats was established (Black, 1900). In 1880, a law was passed which forbade the export of Angora goats from Turkey to South Africa (Black, 1900; Pringle, 1989).In 1896, however, an agreement was made between South Africa and Turkey that a final shipment of Angora goats be made and 33 bucks and 30 does were imported to South Africa (Black, 1900).

Hayes (1868) stated that the reason that the Angora goats were able to survive and produce effectively in South Africa is the resemblance of the landscape and climatic conditions to Ankara. There were further importations reported to have come from Asia and England, and most present day flocks can be traced back to these original animals (Pringle, 1989).

1.3 Present day South African Angora goat production systems

Angora goat farms in South Africa are clustered in the Karoo of the Western and Eastern Cape provinces, where production systems operate under extensive or semi-extensive conditions. Animals are kept in large herds (1000 to 2500 goats) on large open pastures and most farmers maintain both a stud and commercial herd (personal communication – Dr M.A. Snyman, Grootfontein Agricultural Development Institute, Middelburg, National Department of Agriculture Forestry and Fisheries, Eastern Cape, South Africa). As

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with most small stock production systems, artificial insemination (AI) is scarcely used and most breeders opt for natural mating. Most Angora goat breeders practice group mating, flock mating or a combination of the two. Group mating involves keeping mating herds in the ratio of approximately 25 to 35 ewes per buck, where flock mating increases the numbers of animals in mating herds of 200 ewes to four to six buck. In order to ensure maximum successful mating in the herds, Angora breeders often make use of overmating. This method incorporates entire flock mating with group mating towards the end of the breeding season. This increases the likelihood that all ewes that did not fall pregnant after the initial group mating will be pregnant at the end of the mating season. The paternity of the lambs born from the overmated ewes is therefore unknown (Friedrich, 2009). Furthermore, Angora does are renowned for being poor mothers, often abandoning their young at birth, while some does steal kids (Bolormaa *et al.*, 2008). Consequently both maternal and paternal records are often incomplete or inaccurate. It would, however, be impractical (if not impossible) for farmers to improve these records through management alone given the dynamics of small stock breeding practices.

In a previous study by Friedrich (2009), it was found that 25% of the pedigrees recorded by Angora goat breeders were inaccurate or incomplete. This can have serious consequences on selection accuracy as breeders would be selecting breeding parents (particularly sires) based on the performance of their recorded offspring, which is likely to be incorrect. Selection practices by South African Angora goat breeders are generally done using phenotypic values. Some breeders have started making use of selection indices (with animals' own performance values) compiled and documented by Snyman *et al.* (2010). The performance testing scheme for Angora goat breeders is available through the National Small Stock Improvement Scheme (NSIS) at GADI, Middelburg. There has been limited participation of Angora goat breeders in the national scheme, even though there are over 800 breeders in the country (personal communication – Dr M.A. Snyman, GADI, Middelburg, National Department of Agriculture Forestry and Fisheries, Eastern Cape, South Africa). In order for South Africa to maintain its competitive position in the global market, it is vital to prioritise genetic improvement and take all possible measures to improve the quality of the national mohair clip. Constructing sound pedigree records is the first step in this direction as all genetic improvement strategies are built on this foundation. There are several commercial laboratories available in South Africa that perform DNA-based parentage testing. These are included in ADDENDUM B.

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1.4 Aim of the study

The challenges facing Angora goat breeders may be overcome through the implementation of DNA-based parentage verification. In a previous study by Friedrich (2009) a microsatellite marker panel was designed specifically for use in parentage testing in South African Angora goats. The panel includes a number of markers recommended by the International Society of Animal Genetics (ISAG) for parentage studies in goats. Parentage testing using DNA markers has been done routinely in a number of different livestock species, all for the purpose of improving the accuracy if selection in the different production systems.

Selection using EBVs has been proven to improve selection accuracy over the use of phenotypic values. A number of farmers keep performance records of their animals which may be analysed by the National Department of Forestry and Fisheries of South Africa for the estimation of EBV for their animals. The models that were designed and tested for use in the performance testing scheme were used for the estimation of EBVs of breeding sires for various traits of economic importance for Angora goats. These EBVs will be used to illustrate the detrimental effect that pedigree inaccuracies have on selection decisions made by breeders.

The aim of the study was to determine the effect that pedigree inaccuracies can have on selection accuracy and genetic progress in South African Angora goat production systems.

The objectives of the study were to:

- Validate the microsatellite marker panel (consisting of 14 microsatellite markers) designed in a previous study by Friedrich (2009) in a larger population of South African Angora goats.
- Determine the extent of errors present in pedigree records of a prominent South African Angora goat breeder.
- Estimate the EBVs of all sires included in the breeding herd twice: firstly using the pedigrees supplied by the breeder and, secondly, using the DNA-verified pedigrees.
- Evaluate how the estimation of breeding values using inaccurate pedigrees affects the ranks of sires for seven economically important traits for mohair production.

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CHAPTER 2: Literature Review

2.1 Introduction

Parentage verification is the identification of a paternal or maternal match using genetic information of the offspring and either (or both) parents (Cercueil *et al.,* 2002). It has been widely performed in human, plants, animals and even microbiological populations (Jeffreys *et al.,*1985; Geldermann *et al.,* 1986; Broyles & Wyatt, 1990; Jarne & Lagoda, 1996). A number of DNA technologies and statistical methodologies have been developed in order to improve the success and provision of these tests. Parentage is assigned using maximum likelihood estimations (Marshall *et al.,* 1998), by exclusion of non-parents (Dodds *et al.,* 2005) or by fractional allocation (Devlin *et al.,* 1988; Jones & Ardren, 2003). Since the initial implementation of parentage verification in livestock in the 1960's, the methodology and technology used has evolved considerably into what it is today.

Parentage testing in domestic animals was first investigated in cattle in 1940, where the primary concern was preservation of the purity of different breeds (Stormont, 1967). Currently it is being practiced in many different domestic and wildlife species for different applications. In livestock the most important function is the improvement of selection accuracy and genetic progress that requires sound pedigree records (Geldermann *et al,*. 1986; Visscher *et al.*, 2002; Dodds *et al.,* 2005, Pollak, 2005; Van Eenennaam *et al.,* 2007).

The aim of this literature review is to investigate the importance of parentage verification in genetic improvement and selection of livestock, with reference to the available methods performed and applied in different animal species.

2.2 Evolution of DNA Markers in parentage testing

The earliest methodology of parentage testing made use of blood typing, where blood protein antigens served as an indication of the genetic differences between individuals (Silver, 1989; Hines, 1999; Bowling, 2001). However, the drawbacks associated with the use of blood groups created the need for higher precision testing, leading to the development of DNA markers in individual identification and parentage testing (Silver, 1989; Visscher *et al.,* 2002).

The use of DNA markers was developed for parentage testing primarily due to the higher precision achieved compared to blood typing. Moreover, PCR and automated genotyping has become more cost

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effective and less labour intensive (Beuzen *et al.,* 2000; Ozkan *et al.,* 2009). The opportunity to use biological samples (blood, hair, vaginal swabs etc) that may be stored for long periods of time, as well as non-invasive sampling (milk and semen), have further compounded the advantages of doing DNA-based parentage testing over blood typing which requires fresh blood (Visscher *et al.,* 2002).

2.2.1 Blood typing

The discovery of the ABO blood groups in early 1900 by Landsteiner, followed by the characterisation of their Mendelian inheritance by von Dingen & Hirszfeld, presented the first opportunity for parentage testing in humans (Landsteiner& Miller, 1925; Silver, 1989). Further refinement of blood groups led to the development of the MN system by Landsteiner & Levine in 1927, followed by the RH-HR system by Levine & Stetson and Landsteiner & Weiner in 1940. The combination of the ABO, Rh and MN systems was prepared by Kelly, Duffy & Kidd in the 1950's, which culminated in the application of HLA tissue types for the depiction of parentage testing in the 1960's (Silver, 1989). These tests provided the most proficient testing available at the time, giving a maximum potential power of exclusion of a non-parent of 92.44%, leaving no less than 7.56% margin for error (Silver, 1989). The first studies on blood groups for parentage in livestock were in cattle in the 1940s and '50s (Ferguson, 1941; Stormont & Cumley, 1943; Stormont, 1967; Hines, 1999).

Blood typing proved to be a simple and convenient method as results were easily obtainable and simple to interpret, however, it had limitations. Analyses could only be carried out in selected laboratories as commercial kits were not available. Fresh blood was also required for the analyses; therefore samples could not be stored for long periods of time. This prohibited testing animals after their death and transporting samples across countries (Bowling, 2001; Visscher *et al.,* 2002). Furthermore, the possibility that two unrelated individuals may share the same blood group often produced inconclusive results. This presented the need for the development of more refined techniques for verifying pedigree records in humans as well as livestock. The use of the ABO blood group system alone provided a probability of exclusion of around 13%. In combination with other systems (RH, MN groups) the maximum exclusion probability reached 90% (Silver, 1989).

2.2.2 First DNA Markers

Initial studies in the development of alternative higher-powered parentage tests made use of allozymes (enzyme variants encoded by structural genes) and DNA markers such as Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNA (RAPDs), minisatellites (including Variable Number Tandem Repeats – VNTRs) and DNA fingerprinting. (Queller *et al.,* 1993). The lack in

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variation of allozymes in different species and populations rendered them unsuitable for parentage analyses (Queller *et al.,* 1993). These tests involved long and complicated protocols and were known to produce erratic results that were open to subjective interpretation, as with RAPDs and DNA fingerprinting, where bands produced on polyacrylamide gels varied in intensity, leading to inconsistent scoring (Queller *et al.,* 1993).

During the 1980s, the first parentage verification using RFLP DNA markers, was performed in humans (Silver, 1989; Brown, 2002; Weir, 2003), and in the 1990s, the first RFLP panels were developed for use in livestock (Ozkan *et al.,* 2009). Development of high throughput genotyping made way for more efficient methods with more consistent results (Van Marle-Köster & Nel, 2003). However, RFLPs are dimorphic markers and were found to be unsuitable for pedigree analysis (Jeffreys *et al.,* 1985; Liu & Cordes, 2004). Table 2.1 shows a comparison between the properties of different DNA markers used for studies in parentage testing and other molecular applications.

Table 2.1 Comparisons of different DNA markers and their suitability for parentage verification

DNA marker	Mode of inheritance	Number of alleles per locus	Resolution power	Reference
Allozyme	Codominant	2 - 6	Low	Liu & Cordes (2004)
RFLP	Codominant	2	Low	Gerber *et al* .(2000); Liu & Cordes (2004)
RAPD	Dominant	2	Moderate	Liu & Cordes (2004)
Minisatellite	Codominant	Multiple	High	Liu & Cordes (2004)
Microsatellite	Codominant	Multiple	High	Liu & Cordes (2004)
SNP	Codominant	2	High	Liu & Cordes (2004)

Two classes of DNA markers are currently preferred for parentage testing in research and commercial applications: microsatellites and single nucleotide polymorphisms (SNPs) (Spangler, 2012).

2.2.3 Microsatellites

The first studies in the discovery of repeat motif markers that were later recognised as microsatellites began around 1989 in humans (Weber & May, 1989). Since that time they have been widely used for various applications, including parentage verification, owing to their high level of specificity (Queller *et al.,* 1993; Bowling, 2001; Ozkan *et al.,* 2009; Saberivand *et al.,* 2011), This characteristic stems from the fact that they are highly polymorphic (Webster & Reichart, 2005; Ozkan *et al.,* 2009), a result of the high

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mutation rates, producing a potentially infinite number of alleles and a high level of variability, even within populations (Saberivand *et al.,* 2011). Furthermore, microsatellites are abundant and uniformly distributed throughout the eukaryotic genome (Weber & May, 1989; Queller *et al.,* 1993; Saberivand *et al.,* 2011), making them relatively easy to detect.

The use of polymerase chain reaction (PCR) for microsatellite-based parentage verification allows for automated processing (Van Marle-Köster & Nel, 2003) and rapid results as well as the potential of using small quantities of DNA (Webster & Reichart, 2005). Therefore, DNA may be extracted from any body tissue where it is contained, which makes it possible for animals to be tested in retrospect (Webster & Reichart, 2005; Ozkan *et al.,* 2009).

In parentage tests using microsatellite markers, there is a possibility that a non-parent may match certain progeny by chance. This probability increases with decreasing number of markers (and alleles) in a panel (Hoffman & Amos, 2005; Webster & Reichart, 2005; Kalinowski *et al.,* 2007). In populations of smaller effective size, more microsatellite markers are required in order to achieve the same distinguishing power as in a population of larger effective size (Wang & Santure, 2009). Despite this, microsatellite markers have become the DNA marker of choice for parentage verification for livestock (Geldermann *et al.,* 1986; Visscher *et al.,* 2002; Schlötterer, 2004; Siwek & Knol, 2010; Stevanovic *et al.,* 2010). Microsatellite panels have been developed for use in parentage testing in different livestock species, as presented in **Table 2.2**.

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Table 2.2 Summary of microsatellite panels for parentage verification in different species from various studies

Species	Number of markers	Microsatellite panel	Reference
Cattle	10	BM1824; BM2113; INRA023; SPS115; TGLA122; TGLA126; TGLA227; ETH3; ETH10; ETH225	Rehout *et al.*, 2006 Visscher *et al.*, 2002
	23	ADCYC; BM203; BM888; BM1818; BM1824; BM2113; BM4107; BM4208; BRN; CYP21; ETH10; ETH152; ETH225; INRA23; OarFCB5; OarFCB193; RM006; RM067; SPS115; TGLA94; TGLA122; TGLA126; TGLA22Y	Van Eenennaam *et al.*, 2007
	7	BM4307; BM3205; CSSM004; INRA049; OarFCB5; RM029; BM415	Saberivand *et al.,* 2011
Sheep	16	D5S2; INRA23; INRA49; MAF65; McM42; McM527; OarFCB20; TGLA53; CSRD247; HSC; INRA063; MAF214; OarAE119; OarAE129; OarFCB11; OarFCB304	Rendo *et al.*, 2011
Pigs	10	SW24; S0107; S0068; SW936; SW353; S0386; S0355; SW72; TNFB; S0070	Putnova *et al.*, 2003
Horses	6	HMB1; HMB2; HMB3; HMB4; HMB5; HMB6	Binns *et al.*, 1995
	16	AHT4; AHT5; ASB2; HMS6; ASB17; HTG6; HTG7; HMS2; ASB23; VHL20; LEX003; CA425; HTG10; HTG4; HMS7; HMS3	Bierman, 2010
	18	HTG6; VHL20; HTG10; HTG4; AHT5; AHT4; HMS3; HMS6; HMS7; HMS1; ASB2; ASB17; ASB23; CA425; HTG3; HTG7; LEX3; LEX33	Avdi & Banos, 2008
Dogs	10	C04107B; CXX.2054; CXX.2004; CXX.2001; CXX.2132; CXX.2137; CXX.2146; CXX.2088; CXX.2168; CXX.2175	Koskinen & Bredbacka, 1999
	17	CATA; PEZ03; PEZ05; PEZ06; PEZ08; PEZ12; PEZ20; FHC2010; FHC2054; FHC2079; PEZ10; PEZ11; PEZ13; PEZ15; PEZ16; PEZ17; PEZ21	DeNise *et al.*, 2004
Goats	22	SRCRSP23; INRA063; SRCRSP9; MAF65; OarFCB48; SRCRSP13; MCHII-DR; OarFCB20; OarAE54; INRABERN172; ILSTS011; MAF209; TGLA53; ILSTS005; SRCRSP8; SRCRSP3; ILSTS029; SRCRSP15; INRABERN185; SRCRSP7; SRCRSP5; ETH10	Luikart *et al.,* 1999
	12	BMC5221; BMS357; BM7160; BMS1237; BMS585; BM5004; MB068; BMS332; BMS820; BR6027; BM7228; MB045	Ganai & Yadav, 2005
	9	SRCRSP1; SRCRSP5; SRCRSP8; SRCRSP9; INRA011; ChirUCO2; ChirUCO4; ChirUCO5; ETH10	Jimenez-Gamero *et al.,* 2006
	14	BM1258; OarCP73; RM096; TGLA53; BM1818; LSCV44; ILSTS11; ILSTS029; SRCRSP5; INRABERN172; INRA063; OarFCB020; SRCRSP07; INRABERN185	Bolormaa *et al.*, 2008
	11	BETACAP; INRA005; ILSTS0087; INRA006; INRA063; INRABERN172; ILSTS005; ILSTS011; SRCRSP05; OarFCB48; BM3205	De Araujo *et al.*, 2010
	14	BM1258; OarFCB48, SRCRSP24; BM1818; MCM527; SRCRSP5; INRA63; SRCRSP8; INRABERN192; SRCRSP9; CSRD247; BM7160; BM1329	Friedrich, 2009

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There are a number of microsatellite marker panels that have been recommended by the International Society of Animal Genetics (ISAG) for use in parentage verification in different livestock species as shown in Table 2.3. These panels have been tested and proven in different laboratories (elected by the ISAG committee) for different animal populations, covering different breeds in each species, under standard conditions in each laboratory (www.isag.us).

Table 2.3 ISAG recommended microsatellite panels for different livestock species (www.isag.us)

Species	Number of markers	Microsatellite panel
Cattle	9	BM2113; BM1824; TGLA227; TGLA126; SPS115; ETH225; TGLA122; INRA023; ETH10
Sheep	19	CSRD247; HSC; INRA063; MAF214; OarAE129; OarCP49; FCB11; FCB304; D5S2; INRA005; INRA023; MAF65; MCM527; OarFCB20; SPS113; BM1258; BM1329; BM1818; INRA231
Pigs	15	S0005; S0090; S0101; S0155; S0355; S0386; SW24; SW240; SW857; SW951; SW72; SW936; SW911; S0228; S0227
Horses	9	AHT4; AHT5; HMS6; HMS7; HTG4; VHL20; ASB2; HMS3; HTG10
Goats	16	INRA005; INRA063; MAF65; SRCRSP5; SRCRSP8; SRCRSP24; CSRD247; FCB20; ILSTS87; INRA023; MCM527; SRCRSP23; BM1258; BM1329; BM1818; INRA231

2.2.3.1 Statistical parameters for parentage analyses

A number of parameters are used to describe the efficiency of a marker (on its own) and a marker panel as a whole. These explain how informative and effective a marker or a panel is in inferring parentage in a particular population. The performance of individual markers is characterised by the number of alleles per locus, Probability of Exclusion (P_E), Polymorphic Information Content (PIC), heterozygosity (H_E and H_O) and deviation from Hardy-Weinberg Equilibrium (HWE). The different parameters cannot be interpreted independently as their underlying constituents overlap, and should be compared to one another in order to obtain a complete picture of the performance of a marker. The number of alleles per locus describes the variation of a marker in a particular population. Markers with many alleles per locus are considered to be highly polymorphic, have high mutation rates and are more heterozygous in a particular population (Hoffman & Amos, 2005; Burghava & Fuetes, 2010). An alternative measure, more useful than the number of alleles per locus, may be the allele frequencies at each locus (Altet *et al.,* 2001; Hale *et al.,*

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2012). Ideally for parentage studies, alleles should not be too common in a population (Hale *et al.,* 2012), but rare enough such that parent-offspring relationships might be identified.

The probability of exclusion (P_E) is a statistical measure of the power of a marker to exclude all non-parents when offspring and true parent(s) are genotyped (Webster & Reichart, 2005; Van Eenennaam *et al.,* 2007; Bolormaa *et al.,* 2008; Wang, 2009) or the probability that two unrelated individuals in a population will not share any alleles (Rehout *et al.,* 2006). It depends largely on the number of alleles at each locus, the allele frequencies, and the number of individuals that are genotyped (Wang, 2009; Vandeputte, 2012). In the selection of markers for parentage applications, markers with higher P_E are preferred (Van Eenennaam *et al.,* 2007).

Polymorphic Information Content (PIC) gives an indication of the level of variation (or polymorphism) of a marker in a population (Altet *et al.,* 2001). It is calculated for each locus using the number of alleles and their individual frequencies (Buchanan & Thue, 1998), therefore loci with many alleles usually have a large PIC. However, it is possible for such a locus to have a small PIC in the event that few of the alleles have comparatively large frequencies compared to the majority of the other alleles (Buchanan & Thue, 1998). The PIC for polymorphic markers (such as microsatellites) are larger than biallelic markers (such as RFLPs and SNPs) and are therefore generally desired more than biallelic markers for parentage applications.

Heterozygosity, observed (H_O) and expected (H_E), give an indication of the proportion of heterozygote individuals in the population for each locus. Heterozygotes arise as a result of microsatellite mutations producing new alleles (Bhargava & Feutes, 2010) and provide means to more accurately distinguish between related individuals. Heterozygosity of a locus in a population is dependent on the number of alleles per locus and, hence the rate of mutation of the marker, the distribution of the alleles in the population and the number of individuals genotyped (Bolormaa *et al.,* 2008; de Araujo *et al.,* 2010). It is calculated similarly to PIC, using the different alleles and their frequencies at each locus. Generally the observed heterozygosity is lower than the expected heterozygosity (Maruyama & Fuerst, 1985). Very large differences between observed and expected heterozygosity arise as a result of non-random mating, one of the violations of HWE (Wheeler *et al.,* 2003).

Markers may deviate from Hardy-Weinberg Equilibrium (HWE) for a number of reasons, firstly if the population is small and inbreeding occurs or if the marker is under direct or indirect selection (Wang, 2007; de Araujo *et al.,* 2010), or secondly, if null alleles or allelic dropout occur (Hoffman & Amos,

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2005; de Araujo *et al.,* 2010). This may usually be confirmed by referring to the heterozygosity at a locus. A locus that deviates from HWE significantly will likely have a low heterozygosity or show a relatively higher null allele frequency in a population.

Often the average values of these parameters are estimated over all the markers in a panel to evaluate the suitability of a panel as a whole for application in parentage testing. Possibly the most important statistical parameter for a marker panel is the Combined Probability of Exclusion (CPE). It is calculated using the probability that an unrelated sire will match the genotypes of the offspring at all loci included in the paternity test (Webster & Reichart, 2005; Vandeputte, 2012). Microsatellite marker panels should have the potential ability to exclude a non-parent sire from a group of putative sires with up to 99.99% accuracy (Webster & Reichart, 2005). The average PIC of a marker panel can also be taken into account when considering the level of variation of the panel in a population and, hence, how informative a marker panel is in inferring parentage.

Another parameter that characterizes the strength of parentage by a microsatellite marker panel is the LOD score. It is defined as the natural logarithm of the combined likelihood ratio (Meagher, 1986), where the likelihood ratio is the probability of assigning paternity of one putative sire in a population relative to that of another putative sire in the same population (Marshall *et al.,* 1998). The LOD score is calculated as the difference in likelihood ratios between the most likely sire and the second most likely sire. These likelihoods are calculated based on marker allele genotypes and allele frequencies within the population (Marshall *et al.,* 1998). In parentage testing, a LOD score of zero indicates inconclusive parentage, such that the hypothetical sire is equally likely to be the sire as any randomly selected sire in the population. A negative LOD score, usually taken as less than negative three, indicates non-parentage (the candidate sire is less likely to be the true sire owing to the sharing of recurrent alleles and mismatches between sire and offspring at one or more loci). Positive LOD scores imply that the candidate sire is more likely to be the true sire than any other sire in the population (Slate *et al.,* 2000). The LOD score threshold values are summarised in Table 2.4.

Table 2.4 LOD scores and parentage analysis (Slate *et al.,* 2000)

LOD score	Parentage assignment
Less than -3	Parentage rejected
Zero	Inconclusive
More than +3	Parentage confirmed

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The limitation of relying on LOD scores alone as an indicator of paternity occurs when there are related individuals within the putative sire group and no maternal genotypes are available. In such an instance the risk exists that the wrong relative will be assigned as the true father, as the related sires may share alleles at different loci (Marshall *et al.,* 1998).

A potential source of inaccuracy in parentage assignment is the presence of microsatellite null alleles and genotyping errors (Marshall *et al.,* 1998; Dakin & Avise, 2004). Null alleles are those alleles at any microsatellite locus that do not amplify through PCR (polymerase chain reaction) (Dakin & Avise, 2004) and is generally indicated by a heterozygote deficiency. The presence of null alleles may potentially impair the accuracy with which parentage is inferred by causing the false exclusion of non-parents. Genotyping errors have also been shown to erroneously exclude parents as non-parents. Genotyping errors may originate from contamination, microsatellite stutter, null alleles or human error and may have a marked effect on accuracy of parentage assignment, irrespective of the number of markers or alleles employed (Kalinowski *et al.,* 2007).

2.2.3.2 Software and analyses for parentage

A number of statistical software programmes have been developed for parentage analyses. Microsatellite Toolkit (MS Toolkit) of Park (2001) is widely used for the analysis of microsatellite marker panels. Parameters measured by MS Toolkit include the number of alleles per locus, allele frequencies, PIC, P_E, H_O and H_E and deviation from HWE (Park, 2001). It is a free download programme that operates as an add-on to Microsoft Excel.

Programmes that conduct parentage analyses from genotype data are readily available for download on most operating systems. Some such programmes are: CERVUS (Marshall *et al.,* 1998), PARENTE (Cercueil *et al.,* 2002), NEWPAX (Amos, 2000), PROBMAX (Danzmann, 1997) and FAMOZ (Gerber *et al.,* 2003). The different programmes are geared towards different types of studies. For example, FAMOZ can be used for dominant markers (such as RAPDs and DNA fingerprinting bands), codominant markers (such as microsatellites and SNPs) and cytoplasmic markers (for specific inference of paternal or maternal parentage) (Gerber *et al.,* 2003). PROBMAX can also analyse dominant and codominant markers and correct for possible mis-scored alleles (Danzmann, 1997). PARENTE can assign parental pairs simultaneously and can operate over more than one generation (as it takes birth dates into account) (Cercueil *et al.,* 2002). The most widely documented implemented programme used for parentage studies is probably CERVUS. This programme operates on Windows and was designed for paternity inference using maximum likelihood methodology (Marshall *et al.,* 1998; Slate *et al.,* 2000). Codominant marker

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parameters are also computed, including the LOD score for each parentage assignment (Slate *et al.,* 2000). A significant advantage of CERVUS is the correction that can be made for null alleles, genotyping errors and missing paternal genotypes (Marshall *et al.,* 1998; Kalinowski *et al.,* 2007), three factors that could greatly compromise parentage assignment.

2.2.4 Single Nucleotide Polymorphisms

SNPs are biallelic markers (Vignal *et al.,* 2002), making them less informative than microsatellites (Queller *et al.,* 1993). In a study by van Eenennaam *et al.* (2007) on parentage verification in US beef cattle, the efficiency of a microsatellite panel of 23 markers was compared to that of a SNP panel consisting of 28 markers. It was found that the microsatellite panel was more successful in assigning parentage than the SNP panel, where the SNP panel failed in assigning one single sire to each offspring and assigned multiple possible sires. The results of the study suggested that a panel consisting of 40 SNP would not be sufficient for parentage studies (Van Eenennaam *et al.,* 2007). This was confirmed by a study was conducted on dairy cattle in New Zealand by Fisher *et al.* (2009), using different sized SNP panels for parentage testing. The efficiency and success of parentage assignment of different sized SNP panels was compared with a microsatellite panel consisting of 14 markers. SNP panels of less than 40 markers were not successful in parentage assignment. Panels ranging from 40 to 60 SNPs only had better success than the microsatellite panel when used in conjunction with available on-farm records such as birth-calving and mating data. This may be potentially useful in the event that farm records are 100% correct, however, in larger herds, this may be an unrealistic expectation. The study showed that efficiency ratio of number of SNPs needed per microsatellite marker for similar efficiency rate is 5-6 SNPs for every microsatellite (Van Eenennaam *et al.,* 2007; Fisher *et al.,* 2009).

Recently focus has gravitated towards SNP markers for their use in genomics owing to their abundance in the genome and the cost-effective approach of genotyping thousands of these markers in one single array (Sodhi & Schook, 2011). This methodology allows multiple application analysis where the results of a genotyping assay may be used to analyse parentage, disease screening (BLAD, CVM and DUMPs), major gene screening (myostatin or A1/A2 β casein) as well as estimation of Genomic Estimated Breeding Values (GEBVs) (Rothschild & Plastow, 2007; Mullen *et al.,* 2013). One advantage that SNP genotyping has over microsatellites is the high reproducibility with SNPs (Mullen *et al.,* 2013).

ISAG has recommended SNP panels for parentage testing in different species. A minimum of 100 SNP markers are recommended accurate parentage allocation (www.isag.us). The implementation of SNP parentage testing may, however, present a number of challenges. Firstly, all parental animals will be

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required to be genotyped with the parentage SNP panel, which is costly, and may not be possible if the parents are dead and no DNA is available for genotyping. McClure *et al.* (2012) investigated methodology of imputing parental SNP genotypes from microsatellite marker genotypes. This may facilitate the transition from microsatellite-based parentage assays to SNP panels; however the presence of null alleles and genotyping errors in microsatellite genotypes may lead to the construction of incorrect haplotype structures for the inference of SNP genotypes (McClure *et al.,* 2012). The use of SNPs for parentage verification may be worthwhile if genotypes for parentage testing are obtained from a whole genome assay, but not in isolation.

SNP marker panels (commonly referred to as a "SNP chip") have been developed and are commercially available. They differ from microsatellite markers in that the laboratory processing is far less, but the statistical data analysis is far more laborious. SNP chips are comprised of a number of wells containing all SNP markers included in the panel. There is a well for each sample to be genotyped. SNP chips vary in size from the low density 3000 markers (3K chip) to 777 000 markers (HD chip) Bovine SNP chip. There are a number of markers within each chip that are specifically suited for parentage testing, as indicated in Table 2.5 (www.illumina.com; www.affymetrix.com).

Table 2.5 Commercial SNP chips with SNPs contained for parentage verification (www.illumina.com; www.affymetrix.com)

Species	SNP chip	SNP chip size	Parentage-specific SNPs
Cattle	GoldenGate® Bovine 3K Genotyping BeachChip	2900	>100
	Bovine SNP50 Genotyping BeadChip	54 609	
	Axiom® Genome-wide BOS 1 Array plate	>640 000	
	BovineHD Genotyping BeadChip	>777 000	116
Sheep	Ovine SNP50 BeadChip	54 241	138
Pigs	Porcine SNP60 BeadChip	64 232	
Horses	Equine SNP50 Genotyping BeadChip	54 602	
Chickens	Chicken 60K iSelect BeadChip	57 636	
	Axiom® Genome-wide Chicken Array	>580 000	
Goats	50K International Goat SNP chip	53 347	

The SNP chips mentioned in Table 2.5 are available from Illumina™ and Affymetrix™, with the exception of the chicken and goat SNP chip, for which consortia chips are available (Groenen *et al.,* 2011;

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Tosser-Klop, 2012). The Illumina™ SNP chips for cattle and sheep have been validated for application in parentage verification and contain parentage-specific markers within the panel (www.illumina.com).

2.3 Significance of parentage verification in livestock breeding

The necessity for accurate recording of parentage in livestock production systems places pressure on management systems. It is not always possible to carry out single sire mating, observe all parturitions or to separate mothers during birth in commercial livestock herds. Therefore, parentage records are expected to contain a degree of uncertainty. Pedigree errors reported in livestock herds of different species in various countries around the world are as follows: 10% in UK dairy herds (Visscher *et al.,* 2002); between 4 and 23% in German dairy herds (Geldermann *et al.,* 1986); 12% in dairy herds of the Netherlands (Bovenhuis & Van Arendonk, 1991); 9.8% in beef cattle herds in the USA (Pollak, 2005); up to 15% in sheep (Dodds *et al.,* 2007) and 25% in South African Angora goat herds (Friedrich, 2009).

A decrease in selection accuracies was reported by Pollak (2005), where errors existed in recorded pedigrees, along with introduced bias in estimation of genetic parameters, including underestimation of heritability and direct-maternal correlations (Visscher *et al*., 2002). Potential decline in response to selection of up to 2 – 3% has been reported by Visscher *et al.* (2002) regarding selection using EBVs. Banos *et al.* (2001) estimated a decrease of 11 – 15% in genetic improvement in dairy cattle in the United Kingdom on account of parentage errors.

Sound pedigree records are an integral element of successful livestock breeding programmes (Ganai & Yadav, 2005). The estimation of EBVs for one individual makes use of performance data of all the individual's relatives in a population and, therefore, unsound pedigrees can introduce bias into the estimation of these EBVs. Best Linear Unbiased Prediction (BLUP), Restricted Maximum Likelihood (REML) and the Animal Model, all operate under the assumption that reported pedigrees are accurate (Banos *et al.,* 2001; Visscher *et al.,* 2002; Ganai & Yadav, 2005).

2.3.1 Genetic parameters

The estimation of genetic parameters makes use of a relationship matrix and, therefore, the relationships in this matrix should be accurate in order to obtain accurate estimates of these parameters (Sorensen & Kennedy, 1983). Errors in parentage records have led to biased estimation of genetic parameters, contributing to inaccurate EBV estimation. (Geldermann *et al.,* 1986; Banos *et al.,* 2001; Senneke *et al.,* 2004; Ganai & Yadav, 2005; Rehout *et al.,* 2006).

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2.3.1.1 Heritability

The greatest effect of pedigree errors realized is the downward bias (underestimation) of heritability (h^2) for traits under selection (Israel & Weller, 2000; Banos *et al.,* 2001; Visscher *et al.,* 2002). If the heritability of a trait is calculated based on resemblance (or correlation in performance) between parents and offspring, then incorrect parent-offspring matches will portray a weaker association than between offspring and the true parent (Charmantier & Reale, 2005).

Underestimation of heritability attributes a lower proportion of the phenotypic variation to the additive genetic variation (what is contained in the DNA of an individual) (Falconer & Mackay, 1996; Milner *et al.,* 2000). In the study of Lee & Pollak (1997), direct and maternal heritability was underestimated in the presence of 20% paternity errors in beef cattle. Greater bias in heritability estimation was realised by Charmentier & Reale (2005) when population sizes were smaller than in larger populations, owing to greater standard error realized (and possibly higher inbreeding levels) with smaller population sizes in wild birds. Furthermore, they discovered (upper and lower) threshold population sizes above and below which, respectively, no further deviation due to mispaternity in heritability estimate was observed. Greater measures of parentage errors had greater effects on heritability estimate (Van Vleck, 1970; Charmantier & Reale, 2005).

Loss in response to selection is a function of the number of progeny belonging to a sire, heritability of selected traits and paternity error rates (Visscher *et al.,* 2002; Rehout *et al.,* 2006). An estimated decrease in genetic gain of 3% with an error rate of 10%, h^2 of 0.25 and 50 progeny per sire was reported by Visscher *et al.* (2002) in UK dairy cattle. Sanders *et al.* (2006) found that the effect (loss in potential genetic gain) of incorrect recorded paternity was greater in traits with lower heritability in Angeln Dairy cattle in Germany. This is because a larger weight is given to information from relatives than the animal's own phenotypic performance in estimation of EBVs for traits with lower heritability (Long *et al.,* 1990).

2.3.1.2 Direct-maternal genetic correlations, direct-maternal covariance and standard deviation of EBVs

Other less prominent parameters affected include direct-maternal correlations and standard deviation (SD) of sire EBVs. Sire misidentification may cause negative direct-maternal correlations to become positive, which would result in decreased estimates of both direct and maternal heritability (Lee & Pollak, 1997; Senneke *et al.,* 2004; Rehout *et al.,* 2006) and inflated maternal component of heritability (Lee & Pollak, 1997; Senneke *et al.,* 2004; Rehout *et al.,* 2006). The contribution of permanent maternal environmental effect to the total variance increased for birth weight and weaning weight for USA beef cattle as the

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proportion of misidentified progeny decreased (Senneke *et al.,* 2004), thus reducing the contribution of additive genetic variance to the total genetic variance. Lee & Pollak (1997) established sire misidentification as a source of bias in estimation all genetic parameters involved in genetic evaluation of weaning weight in beef cattle, with prominent effects in direct covariance (covariance becomes less negative with sire misidentification).

2.3.2 EBV estimation and sire ranking

Misidentification of parentage, particularly paternity, induces bias in the EBV calculation of breeding animals (Long *et al.,* 1990; Banos *et al.,* 2001; Senneke *et al.,* 2004). The effect is most pronounced in very high and very low merit sires (Banos *et al.,* 2001), with the EBV biased toward the mean (higher merit sires appear less superior and lower merit sires appear less inferior) and a resulting effect of reducing overall variation in the population (Geldermann *et al.,* 1986; Israel & Weller, 2000). The consequence of this is that high merit progeny could be wrongly assigned to inferior sires, resulting in inaccurate selection of supposedly superior animals, often favouring younger unproven sires (Israel & Weller, 2000). Breeders use sire ranking as a method of selecting the most superior sires in the herd. Sires may be ranked on their own phenotypic performance or on EBVs for each trait or in a selection index. Ranking using EBVs is the most affected by pedigree errors (Long *et al.,* 1990).

EBV estimation and accurate sire ranking is crucially important for international comparisons of sires, as is the case in the dairy industry where semen is marketed globally. Paternity errors of dairy cows resulted in biased estimates of genetic correlations and international conversions between countries. Bias in these evaluations creates a tendency for breeders to select domestic bulls over international bulls, which limits international exports and the potential genetic gains from a larger parental gene pool (Banos *et al.,* 2001). It is here that genetic parameters such as genetic correlations between countries and sire standard deviation play an important role in this regard. Genetic correlations between countries are interpreted as the interaction of genotype with environment (GxE) (Banos *et al.,* 2001). Lower correlations indicate a high GxE and are not desirable when selecting foreign sires. Correlations between the USA and other countries have decreased by 0.04 to 0.06 in populations containing 11% recorded paternity errors (Banos *et al.,* 2001). According to Banos *et al.* (2001), sire SD affects the slope of EBV conversion from one country to another and disrupts the ranking of bulls. Estimations of SD may decrease up to 8 – 9% with 10% pedigree error rate (Banos *et al.,* 2001). In the study of Van Eenennaam *et al.* (2007) the effect of incorrect parentage on sire ranking was investigated, where sires that were (erroneously) placed in the top ten performing sires did not, in fact, sire any offspring at all. In this case, superior sires were rated more poorly than their true value. The study showed that, of these ten sires (that did not sire any offspring);

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nine were younger bulls, confirming that decisions based on inaccurate pedigrees favour younger unproven sires (Israel & Weller, 2000).

The incorporation of inaccurate pedigrees into selection programmes has realised a potential shortfall in the genetic progress made compared to that which is expected. Visscher *et al.* (2002) estimated a decrease in genetic gain of 3% in UK dairy cattle with a 10% parentage error rate, as well as decrease in genetic trend for milk yield of 11% for cows and 14% for bulls with 11% paternity discrepancy. Similar findings were presented by Israel & Weller (2000) for Israeli dairy cattle and Jimenez-Gamero *et al.* (2006) for dairy goats in South-eastern Spain with up to 4% decreased genetic progress. Banos *et al.* (2001) reported a decrease of estimated genetic trends of 11 – 15% in USA dairy herds.

Parentage testing and pedigree reconstruction is also an important method of assessing population structure. Incomplete knowledge of population structure, both within herd and within breed, can lead to selection of breeding parents that are in some way related. Risk of inbreeding is higher in small populations as the probability that two randomly chosen individuals may be related is much greater than in larger populations (Avdi & Banos, 2008). In populations where inbreeding levels are no longer controlled, inbreeding depression occurs, and animals may display decreased fitness levels and reproductive ability as well as lower survivability (higher involuntary culling) and general performance (Sørensen *et al.,* 2005; Mrode *et al.,* 2009). Inbreeding is associated with lower levels of variation (increased homozygosity), which may have a stunting effect on genetic gain as well as the ability of animals to evolve and adapt to changing environments (Avdi & Banos, 2008). Very small populations with excessive inbreeding levels may reach the point of a genetic bottleneck (Sørensen *et al.,* 2005). These populations may seem large by population census, while the effective population size (Ne) is far smaller than it appears (Sørensen *et al.,* 2005).

2.4 Application of parentage verification in different livestock species

Parentage verification has application in selection of all livestock as genetic analyses rely on the confidence of pedigree records. ISAG (International Society of Animal Genetics) has recommended parentage verification microsatellite panels for various species mentioned and commercial kits are available for purchase from biotechnology-based companies (www.appliedbiosystems.com/). A summary of the recommended microsatellite panels available for different species was given in Table 2.2. Commercial SNP chips for genomic selection are produced and marketed by Illumina™ and Affymetrix™, where a number of SNP markers have been identified specifically for parentage verification purposes; these have been listed in Table 2.5 (www.illumina.com; www.affymetrix.com).

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2.4.1 Dairy cattle

Dairy cattle parentage verification is concerned mainly with paternity testing as the chance of dam misidentification is negligible when compared to that of the sire; furthermore greater genetic gain is realised through sire compared to the dam selection (Israel & Weller, 2000). Even with the extensive use of AI in the dairy industry there is a possibility for parentage errors, including incorrect recording of inseminations and consequent overmatings, misdiagnosis of pregnant animals as being unpregnant and semen handling errors by the AI technician and labelling errors at the factory (Visscher *et al.,* 2002). In the event that producers cannot financially afford to genotype their entire herd, priority for parentage testing should be given to those cows that take part in progeny tests for progeny proven bulls (Israel & Weller, 2000).

The dairy breeding industry, being globally based, relies on progeny testing for genetic improvement and across-country evaluations, therefore, correct sire identification is crucial (Geldermann *et al.,* 1986; Banos *et al.,* 2001; Visscher *et al.,* 2002). Given the large group of putative sires and the possibility of errors on AI and herd level, it may not always be possible to assign parentage to all animals through exclusion of non-parents. In cases where a cow's parentage is somewhat uncertain, rather than assume parentage of the cow, it may be safer to leave it unassigned (Visscher *et al.,* 2002). Correct pedigree records are also crucial in the dairy industry because of the extensive use of top-ranking bulls internationally (Mrode *et al.,* 2008), which increases the probability that sequentially selected sires in a cow herd may be related. This should be avoided in order to limit the level of inbreeding in the herd, as discussed previously. Pedigree error rates for Holstein herds across different countries are given in Table 2.6.

Table 2.6 Holstein pedigree error rates across countries

Breed	Country	Parentage error rates	Reference
Holstein	Worldwide	~25%	Banos *et al.,* 2001
	UK	10%	Visscher *et al.,* 2002
	Netherlands	12%	Banos *et al.,* 2001
			Rehout *et al.,* 2006
	Denmark	5 – 15%	Israel & Weller, 2000
	Germany	23%	Rehout *et al.,* 2006

2.4.2 Beef cattle

In the beef industry, some breeders make use of AI, but there is still a large proportion of herds (especially commercial herds) that breed on open ranges with multiple sires (often 10 – 15 bulls per

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camp) (Pollak, 2005; Gomez-Raya *et al.,* 2008; Kios *et al.,* 2012). A significant hindrance to genetic progress in the beef industry is inaccuracies in pedigree recording, which is especially important in the seed stock sector (Pollak, 2005). An error rate in parentage assignment of 9.8% in beef cattle herds in the USA was reported by Pollak (2005).

Breed specific marker panels have been developed and across-breed ISAG microsatellite panels are recommended and may be applied to all breeds within the species (Pollak, 2005; FAO, 2011). Approximately 2400 microsatellite markers have been mapped to the bovine genome (http://dga.jouy.inra.fr/cgi-bin/lgbc/summary.operl?BASE=cattle), providing a vast selection of markers from which to choose the most suitable for parentage analysis. Higher resolution panels are required in the seed stock sector than in commercial herds, because of the greater number of progeny and other pedigrees that stem from this sector (Pollak, 2005). SNP chip panels have been produced for beef and dairy cattle (Bovine SNP chips) and are available in different densities as indicated in Table 2.5.

Senneke *et al.* (2004) reported that sire misidentification introduced bias to the estimation of genetic parameters for weaning weight and birth weight in Hereford cattle, particularly on direct-maternal genetic correlations. This effectively resulted in biased EBV estimations and genetic evaluations. The study suggested that the use of DNA-based parentage testing would have a favourable effect on national genetic evaluations and genetic gain. Pollak (2005) reported that the USA beef breeders in both seed stock and commercial sectors have embraced the implementation of DNA technologies and with the advancements of different DNA parentage testing methodologies and improved cost-effectiveness of genotyping, this is only expected to improve.

It may be worthwhile to carry out a return on investment (ROI) or cost-benefit analysis for beef breeders in order to evaluate potential returns that may be realised through the routine implementation of parentage testing. (Senneke *et al.,* 2004; Pollak, 2005; Gomez-Raya *et al.,* 2008). Factors that affect the benefit to cost ratio for paternity tests include the number of bulls in the herd and number of markers in the panel, with the bull culling rate having the largest effect on the profitability of a DNA parentage testing programme (Gomez-Raya *et al.,* 2008).

2.4.3 Small stock

Many sheep and goat breeds are popular among commercial and subsistence farming sectors owing to their ability to adapt to harsh climates (El Aich & Waterhouse, 1999). Goats are especially abundant in poorer, rural areas worldwide (Luikart *et al.,* 1999; De Araujo *et al.,* 2010), where animals are largely

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grouped with males and females together on grazing lands and usually no pedigree records exist (Siwek & Knol, 2010).

Small stock herds, particularly goats in African regions, are generally much larger in number than other livestock species and breeders often make use of group mating practices as well as overmating in commercial systems (Friedrich, 2009). These mating practices place restrictions on accurate recording of pedigrees (Ganai & Yadav, 2005; Friedrich, 2009). Pedigree errors may be as high as 15% in sheep (Dodds *et al.,* 2007), 25% in Angora goats (Friedrich, 2009) and up to 23% in Indian goat breeds (Ganai & Yadav, 2005). Furthermore, some small stock breeds, such as Angora goats, are known to have weak mothering abilities and tend to abandon their young at birth, resulting in maternal as well as paternal discrepancies (Bolormaa *et al.,* 2008). These pedigree errors have resulted in losses of genetic gain (Dodds *et al.,* 2007) and inaccuracies in sire rankings (Ganai & Yadav, 2005).

Genetic progress in the small stock sector would present great potential benefits to developing countries as well as producers in the commercial industry. Sheep and goats provide a variety of resources to people including meat, milk, fleece and hides in both subsistence and commercial capacities (Casey, 1992; Boutonnet, 1999; Mann, 2000; Vizard, 2000; Haenlein, 2004; Haenlein & Wendorff, 2006). Improving the production of these commodities through genetic selection will benefit the economy and the wellbeing of people worldwide (Vizard, 2000). However, molecular research in small stock lags heavily behind that of other livestock species, more so in goats than sheep (Visser, 2011). There have been around 1400 microsatellite mapped on the bovine genome and only close on 400 exist for goats (Maddox & Cockett, 2007). A consortium SNP chip has been developed for goats (Tosser-Klop, 2012).

2.4.4 Pigs

Generally breeding programmes in pig production systems make use of multiple sires and/or AI for line breeding. This necessitates the use of parentage testing, especially for elite breeding stock (Putnova *et al.,* 2003). For this purpose, microsatellite and SNP panels have been developed for parentage testing across pig breeds (Putnova *et al.,* 2003; Rohrer *et al.,* 2007). Inbreeding is also a major concern in swine due to the extensive use of line breeding. This has created additional requirement for DNA markers that could distinguish between lines of animals; such marker panels consist of 60 SNP or 10 microsatellites, of which microsatellites proved more useful in crossbred than purebred populations as different alleles were present in each breed (Rohrer *et al.,* 2007).

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It may be advisable to include more markers in parentage studies in swine according to Putnova *et al.* (2003), as true P_E values may be lower than expected due to the higher degree of relatedness in breeding herds. The effective population size of pure breeds (as demonstrated in Belgian Landrace) has decreased substantially since the implementation of line breeding, even to the point of risked endangerment (Janssens *et al.,* 2005). Parentage testing and pedigree analyses have been used to study genetic variability and to assist selection of breeding animals for the conservation of this variability. Inbreeding levels of the French landrace stand at 2%, with a yearly change of 0.16%, which was slightly greater than the French Pietrain and Large White (Janssens *et al.,* 2005).

2.4.5 Horses

Parentage testing has been used in the equine breeding industry since the 1980s. Since 1986, Thoroughbred horses in the UK, registered by the general stud book (Weatherbys), had to undergo compulsory parentage testing (Binns *et al.,* 1995). Traditionally all equine parentage testing was conducted using blood groups (Sereno *et al.,* 2008), however, due to inability to solve some cases such as when a mare had been covered by two related stallions, horse breed societies converted to the use of DNA markers (microsatellites) (Binns *et al.,* 1995; Avdi & Banos, 2008).

Horse racing in South Africa enforces that before a foal may be registered with the stud book of the National Horseracing Authority of Southern Africa, parentage of the foal must be verified using either blood typing or DNA-based typing. (The Rules of The National Horseracing Authority of Southern Africa, 2011 - http://www.horseracingauthority.co.za/pubs/docs/rules/Rules_07_2011.pdf). Horse racing in general has strict rules surrounding eligibility of horses that may be registered in the stud book and, therefore, parentage assignment testing methods for these animals are required to be airtight. The ISAG recommended panel of microsatellites for international horse parentage verification has been developed (Table 2.3), furthermore, a recent study by Bierman (2010) assessed the efficiency of a panel of 16 microsatellite markers against that of a 9 marker ISAG panel, where the larger (more recent) panel proved to be more effective (Bierman, 2010).

2.5 Conclusions

Parentage verification is an integral part of livestock breeding programmes. Discrepancies in pedigree errors led to biased EBVs and inaccurate selection as well as excessive levels of inbreeding in livestock populations. This may potentially lead to loss in genetic gains in a number of livestock species. Microsatellite markers have been the DNA marker of choice for the past few decades. Although studies reflect progress in the development of SNP marker panels, microsatellites remain popular because of their

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high polymorphicity. Marker-based parentage is becoming more widely accessible to breeders as the economic efficiency of DNA genotyping has increased. Parentage studies have been done in most livestock species, with the majority of the research directed toward the dairy industry. Therefore, there is scope for further study in other livestock species, with the use of recommended ISAG marker panels, for improvement of pedigrees and greater genetic progress through increased implementation of parentage testing.

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CHAPTER 3: Materials and Methods

3.1 Introduction

In this study, parentage was verified in a population of 381 South African Angora goats in eight half-sib families, a group of unallocated offspring and a group of putative sires. All animals included in the study belonged to one breeder. A panel of 12 microsatellite markers was used for the study. Parentage errors in the breeder's pedigree records were quantified and incomplete pedigrees were amended. The impact of incorrect parentage records on selection accuracy was measured by calculating the Estimated Breeding Values (EBVs) with their accuracies for seven economically important traits for the 21 breeding sires. The sires were then ranked according to the EBVs and the ranks based on the farm-recorded pedigrees were compared with those of the DNA-verified pedigrees. Ethical approval was obtained for the use of biohazardous material (reference number: EC1110018-073) from the Ethics Committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria.

3.2 Materials

3.2.1 Population

The population of Angora goats included in the current study were comprised of eight half-sib families, each with a common sire, as well as a group of unallocated offspring and a group of putative sires. The pedigree records of the half-sib families were obtained from the breeder. No maternal pedigree data was included in the study. A summary of the families included in the study is provided in Table 3.1.

Table 3.1 Summary of the Angora goat families included in the current study

Family	Sire I.D.	Sire name	Number of offspring
A	142/1412	A1	26
B	142/2431	A2	70
C	142/3416	A3	34
D	142/6238	A4	34
E	200/1108	B1	35
F	116/3070	C1	24
G	230/4175	D1	69
H	154/5659	E1	25

In addition to the eight half-sib families, 40 offspring that were not allocated any sire by the breeder, as well as 16 putative sires were included in the study. All possible sires for unknown offspring were included in the study. A summary of all individuals included in the study is presented in Table 3.2.

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Table 3.2 Summary of all individuals included in the study

Individual group	Number of individuals
Total animals in families	325
Unallocated offspring	40
Additional putative sires	16
Total	**381**

Angora goat blood samples for the study were acquired from the GADI-Biobank at the Grootfontein Agricultural Development Institute (GADI, Middelburg, Eastern Cape Province, South Africa). The biobank was established as a biological reserve for improvement and conservation of small stock in South Africa in 2006 (Snyman, 2011). The blood for the biobank was collected by the staff from the GADI (South African Department of Agriculture, Forestry and Fisheries) from farms during routine visits. Blood samples were collected in 10ml EDTA vacutainer tubes and divided into four duplicate aliquots of 2ml each and stored at -80ºC. A total of 16 683 animals' blood samples have been collected and stored in the biobank to date (Snyman, 2011). The Angora goat blood and DNA contained in the bank was contributed by eight different Angora goat producers in the Karoo region of the Eastern Cape and Western Cape Provinces (Snyman, 2011). The population for the current study was selected from three herds of one stud breeder, based on availability of blood, completeness of pedigree and phenotypic data and half-sib family size. The pedigrees recorded were according to the AI and single-sire mating records of the breeder. Putative sires and unallocated offspring were those individuals involved in group mating near the end of the mating season and, therefore have no pedigree records.

The breeder makes use of AI, single-sire mating, group mating and overmating. AI is performed during the first 3 – 5 days of the mating season (usually end of February to early March). All does that are selected for AI are synchronised. Single-sire mating starts at roughly the same time (beginning to mid-March), where a herd of does are placed in a camp together with one buck. Young does entering the breeding herd for the first time are group mated from the beginning of the mating season. All first time breeding does are placed into the camp with all "group" sires (known as "putative" sires in the current study). The does are left with the bucks for two mating cycles (roughly 42 days), after which the bucks are removed for about five days. The mating season is then concluded with a cycle of overmating (roughly 21 days), where all the does and bucks are placed in one camp together. Consequently, kids that are born early in the lambing season (from mid July until September) may have been conceived through AI, single-sire mating or group mating. Kids born from September onwards are most likely to have been

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conceived during the overmating period (personal communication – Dr M Snyman, GADI, Middelburg, Eastern Cape Province, South Africa).

3.2.2 Microsatellite genotyping

Each individual was genotyped with 12 microsatellite markers from the panel designed for South African Angora goats by Friedrich (2009). The marker primers were manufactured by Applied Biosystems International (www.appliedbiosystems.co.za – Life Technologies, Cape Town, South Africa) with custom designated fluorescent labels for allocation into two genotyping sets. The marker details are shown in Table 3.3.

Table 3.3 Characteristics of microsatellite markers: sequences, chromosome number in *Capra hircus* species, annealing temperatures, fluorescent labels used, expected ranges and Genebank accession numbers

Microsatellite	CHI	Annealing Temperature (C°)	Fluorescent Label	Expected range	Genebank accession number
BM 1258	23	58	Pet®	90 – 130	G18385
BM 1329	6q15	55	Vic®	160 – 180	G18422
					AF394444
BM 1818	23	58	6-Fam®	240 – 270	G18391
BM 7160	22	55	6-Fam®	160 – 180	G18819
					UniSTS: 253556
CSRD 247	Unknown	55	Vic®	200 – 260	UniSTS: 251420
HSC	23q22	55	Vic®	260 – 310	L23481
INRABERN 192	7	54	Pet®	170 – 200	X71507
INRA 63	18q22	54	6-Fam®	150 – 170	L34277
MCM 527	7	55	Ned®	150 – 180	L22197
OarFCB 48	17q15	60	Pet®	150 – 170	L22193
SRCRSP 5	21q14	55	Ned®	150 – 190	L22200
					UniSTS: 254136
SRCRSP 8	Unknown	55	6-Fam®	210 – 240	Not available

3.2.3 Phenotypic data

Phenotypic data for economically important traits for each individual in the current study were obtained from records filed at GADI. Traits included in the EBV estimation and sire ranking analysis were fleece traits (second shearing fleece weight and fibre diameter), early body weight traits (birth weight, weaning weight and eight month weight), and mature body weights (yearling weight and 16 month weight). The descriptive statistics for all traits included in the study were calculated for the entire South African Angora goat population born between 2000 and 2009, as routinely analysed in the South African Small Stock Improvement Scheme and are presented in Table 3.4.

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Table 3.4 Descriptive statistics including mean, standard deviation, minimum and maximum values, of the traits used in the current study including fleece weight, fibre diameter, birth weight, weaning weight, eight month weight, yearling weight and 16 month weight, for South African Angora goat kids born from 2000 to 2009 (Personal communication – Dr M. Snyman, GADI, Middelburg, Eastern Cape, South Africa).

Trait	Mean ± SD	Minimum	Maximum
Fleece weight (FW, kg)	1.17 ± 0.35	0.1	3.4
Fibre diameter (FD, µm)	26.06 ± 2.80	2.6	40.7
Birth weight (BW, kg)	3.20 ± 0.53	1.5	5.5
Weaning weight (WW, kg)	16.10 ± 3.96	5.0	30.0
Eight month weight (W8, kg)	20.91 ± 6.08	9.0	44.0
Yearling weight (W12, kg)	20.47 ± 4.84	10.0	52.0
16 month weight (W16, kg)	22.45 ± 4.50	11.0	45.5

3.3 Methods

3.3.1 Molecular component

DNA extraction

Blood samples were transported from the biobank at GADI to the University of Pretoria, where they were stored at 4ºC until extraction. All DNA extractions were done at the Animal Breeding and Genetics Laboratory, Department of Animal and Wildlife Sciences, using the Qiagen DNeasy Blood and Tissue kit® (Qiagen – Whitehead Scientific [Pty] Ltd, Cape Town, South Africa) according to the manufacturer's instructions. Each extraction procedure yielded 200µL aqueous DNA. Following DNA extraction, the blood samples were stored at -40ºC.

Each DNA extraction was followed by an agarose gel electrophoresis to determine a crude estimation of the concentration of the extracted DNA. A solution consisting of 3µL DNA and 2µL loading buffer was loaded into a 1% agarose gel and run on a Hoefer HE 33 Mini Horizontal Submarine Unit® (Amersham Pharmacia Biotech Inc.). The brightness of the bands produced on the gel and viewed over a UV light provided a relative concentration of the yielded DNA.

Microsatellite optimization and PCR amplification

The microsatellite panel designed in a previous study on South African Angora goats by Friedrich (2009) was used in the current study. Each of the 14 markers was tested at the annealing temperatures recommended in the previous study and revised annealing temperatures were determined for each marker where necessary. Four randomly chosen DNA samples from the trial population were used for the

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optimisation of each marker. Reactions were amplified in singleplex. The forward primer for each marker was labelled with a fluorescent dye (Red [PET®], blue [6-FAM®], green [VIC®] or yellow [NED®]) and the markers were arranged into two genotyping sets, with allocated dye colour, according to the respective expected PCR product fragment size ranges. A final panel of 12 microsatellite markers were selected from the original panel of 14 markers based on successful amplification during optimisation. Characteristics of the microsatellite markers are summarised in Table 3.3.

PCR final reactions consisted of a final volume of 15µL.10µL contained the mastermix and primer-taq mix in the following composition: 6.1µL molecular grade distilled water and 3µL Bioline MyTaq® 5x reaction buffer (containing buffer, $MgCl_2$ and dNTPs – Celtic Diagnostics Inc, South Africa), constituting the 9.1µL mastermix, and 0.3µL each of both forward and reverse primers (Applied Biosystems International – Life Technologies, Johannesburg, South Africa) and 0.3µL Bioline MyTaq® enzyme. 5µL DNA, with a concentration of between 60ng/µL and 120ng/µL, was then added to the mixture to make up the total volume of 15µL. PCR amplifications were performed using a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, USA) and a Kyratech SuperCycler®(Celtic Diagnostics – Cape Town, South Africa). The PCR programme included a denaturation step of 10 minutes at 94ºC, followed by 33 cycles of 45 seconds at 94ºC, 80 seconds at the marker’s annealing temperature and 60 seconds at 72ºC, ending with a final extension step of five minutes at 72ºC. Markers which showed elevated levels of non-specific amplification during optimisation were further optimised using a longer final extension step of up to 25 minutes at 72ºC.Each PCR amplification was followed by an agarose gel electrophoresis run to verify the presence of PCR product. A solution containing 3µL PCR product and 2µL loading buffer was run on a 3% agarose gel.

Genotyping

The PCR products of each amplification were diluted in a ratio of 1:10 before genotyping. Each individual was genotyped in two genotyping sets as indicated in Table 3.5.Genotyping was performed using an ABI PRISM® 3100 DNA Genetic Analyser (Applied Biosystems, Foster City, USA) and an Applied Biosystems 3500xL Genetic Analyser (Life Technologies, Carlsbad, US) at the Forestry and Agriculture Biotechnology Institute (FABI) at the University of Pretoria.

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Table 3.5 Microsatellite marker genotyping sets

Microsatellite marker	Set	Size range	Label
BM1258	1	90 – 130	Red (PET™)
OarFCB48	1	150 – 170	Red (PET™)
INRA63	1	150 – 170	Blue (6-Fam™)
BM 1818	1	258 – 270	Blue (6-Fam™)
CSRD247	1	200 – 260	Green (NED™)
MCM527	1	150 – 180	Yellow (VIC™)
INRABERN192	2	170 – 200	Red (PET™)
BM 7160	2	160 – 180	Blue (6-Fam™)
SRCRSP8	2	210 – 240	Blue (6-Fam™)
BM1329	2	160 – 180	Green (NED™)
HSC	2	260 – 310	Green (NED™)
SRCRSP5	2	150 – 190	Yellow (VIC™)

Samples were prepared for genotyping by combining 1µL of the diluted, pooled PCR product with 9µL Formamide-LIZ 500® (Applied Biosystems International – Life Technologies, Johannesburg, South Africa) size standard mix, in the ratio 1000:14 Formamide to LIZ. Allele fragment sizes were analysed using GeneMarker® software (Applied Biosystems, Foster City, USA).

3.3.2 Molecular data analyses

Alleles per locus for each individual were computed using the Microsoft Excel-based MS Toolkit® software (Park, 2001) to determine allele frequencies, polymorphic information content (PIC) and expected and observed heterozygosity (H_E and H_O) for each microsatellite marker. Parentage analyses, and calculation of exclusion probability, LOD and Delta scores and confidence levels, were performed using Cervus 3.0 (Marshall *et al.,* 1998). Results obtained from MS Toolkit® were validated using Cervus 3.0. A parentage analysis consists of an allele frequency analysis, a simulation of parentage analysis and the parentage analysis. The input parameters used in the analyses are summarised in Table 3.6.

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Table 3.6 Input parameters for simulations using Cervus 3.0

Simulation step	Input parameter	Input values
Allele frequency analysis	Number of individuals	381
	Number of loci	12
Simulation of parentage analysis	Number of offspring	360
	Number of candidate fathers	24
	Proportion of candidate fathers sampled	1.0
	Proportion of loci typed	1.0
	Proportion of loci mistyped	0.01
	Relaxed confidence level	80%
	Strict confidence level	95%

The confidence of each parentage assignment was resolved based on Cervus 3.0 generated LOD scores. Upstream analyses were utilised for the calculation of LOD scores by including marker allele frequencies across all markers in the entire population. LOD scores are calculated as the natural logarithm of the likelihood ratio, which is the probability of finding the same genotype at a specific locus in two unrelated individuals in a population (Marshall *et al.,* 1998). The likelihood ratio is calculated by the equation:

$$L(H_{1\,;}\,H_2|D) = \frac{P(H_1)}{P(H_2)}$$

Where$L(H_{1\,;}\,H_2|D)$ is the probability of observing data D under hypothesis P, $H_1$is the hypothesis that the candidate sire is the true sire of an offspring and H_2 is the hypothesis that the candidate sire is not the true sire of the offspring (Marshall *et al.,* 1998; Kalinowski *et al.,* 2007). The LOD score would be expressed as:

$$LOD = \ln\frac{P(H_1)}{P(H_2)}$$

Delta values, denoted by Δ, depicts confidence of LOD scores and is determined by calculating the difference between the LOD scores of the most likely and second most likely sire. The larger this value, the greater the confidence is in the assigned parentage (Kalinowski *et al.,* 2007).

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3.3.3 Data analyses

EBV estimation

The data set used for the estimation of sire breeding values comprises 5077 records of Angora goat kids born from 2000 until 2009. Estimated Breeding Values (EBVs) for each sire were computed using the ASREML programme (Gilmour *et al,* 2002) for traits of economic importance. Traits included in the analysis include birth weight, weaning weight (four month weight), eight month weight, 12 month weight, 16 month weight and two fleece traits, namely second shearing fleece weight and average fibre diameter.

The data was initially analysed by least-squares methods to identify the non-genetic effects which contributed significantly to variation, using the General Linear Model (GLM) procedure of the SAS computer package (SAS, 2009). Variance components were estimated using the ASREML programme of Gilmour *et al.* (2002). Single-trait animal models were fitted for all traits. Direct additive and maternal additive genetic effects, with or without a covariance between them, and maternal permanent environmental effects were used in different combinations to yield six models, which were fitted for all traits to determine the most suitable model for estimation of breeding values.

The six models were:

$\mathbf{y} = X\mathbf{b} + Z_1\mathbf{a} + \mathbf{e}$ 1

$\mathbf{y} = X\mathbf{b} + Z_1\mathbf{a} + Z_2\mathbf{c} + \mathbf{e}$ 2

$\mathbf{y} = X\mathbf{b} + Z_1\mathbf{a} + Z_2\mathbf{m} + \mathbf{e};$ with $cov(a,m) = 0$ 3

$\mathbf{y} = X\mathbf{b} + Z_1\mathbf{a} + Z_2\mathbf{m} + \mathbf{e};$ with $cov(a,m) = A\sigma_{am}$ 4

$\mathbf{y} = X\mathbf{b} + Z_1\mathbf{a} + Z_2\mathbf{m} + Z_3\mathbf{c} + \mathbf{e};$ with $cov(a,m) = 0$ 5

$\mathbf{y} = X\mathbf{b} + Z_1\mathbf{a} + Z_2\mathbf{m} + Z_3\mathbf{c} + \mathbf{e};$ with $cov(a,m) = A\sigma_{am}$ 6

where **y** is a vector of observed traits of animals.

b, **a**, **m** and **c** are vectors of fixed effects, direct additive genetic effects, maternal additive genetic effects and maternal permanent environmental effects respectively.

X, Z_1, Z_2 and Z_3 are incidence matrices respectively relating fixed effects, direct additive genetic effects, maternal additive genetic effects and maternal permanent environmental effects to **y.**

e is the vector of residuals; A is a numerator relationship matrix.

σ_{am} is the covariance between direct additive genetic and maternal additive genetic effects.

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Log likelihood ratio tests were carried out amongst all six models to determine the most appropriate model for each trait (Morrell, 1998). Model 5 was the most appropriate for birth weight, weaning weight, eight month weight and 12-month weight. Model 1 was the most appropriate for 16-month weight, fleece weight and fibre diameter.

Estimated breeding values and accuracies were obtained as back solutions with the ASREML programme (Gilmour *et al*., 2002). EBVs were estimated twice. Firstly with the pedigree records as obtained from the breeders, and secondly with pedigrees according to DNA-based parentage verification after correct offspring have been assigned and previously unknown offspring allocated to correct parents. Previously unallocated offspring were assigned to correct sires. EBVs and accuracies, as well as sire rankings obtained with the two runs were further compared using the Spearman Rank Correlation (Long *et al.,* 1990).

Accuracies of EBVs were calculated as

$$\frac{\sqrt{1-[(\text{predicted error variance reported with each BLUP value})^2}}{\text{additive genetic variance of the specific trait}}$$

Sire ranking

Breeding sires were ranked according to breeding values for each trait evaluated. Sires were initially ranked according to the EBVs estimated using the breeder's pedigree records and then again after restructuring of pedigrees according to DNA-based parentage allocation and revised estimation of sire EBVs. Ranking was done using the Spearman rank correlation method of Long *et al.* (1990).

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CHAPTER 4: Results

4.1 Evaluation of microsatellite marker panel

A microsatellite panel of 14 markers was selected based on the study by Friedrich (2009) on South African Angora goats. Two markers (SRCRSP 9 and SRCRSP 24) were discarded from the original panel due to failure to amplify during the optimisation of the markers. The final panel of 12 microsatellite markers used in the study is shown in Table 4.1. Although performance on this particular panel has already been published, the suitability of the markers for parentage testing was verified again in the present study population. The performance of the markers regarding their suitability for inclusion in a parentage testing panel was based on the following parameters: allele frequencies, total number of alleles, observed and expected heterozygosities (H_O and H_E), Polymorphic Information Content (PIC) and exclusion probability (PE) and null allele frequency (F_{NULL}). Table 4.1 depicts the number of alleles per locus, number of animals genotyped per locus, alleles observed in the population as well as most and least frequent allele with their frequencies.

Table 4.1 Summary of alleles per locus of 12 microsatellite markers for the entire population

Locus	k	N	Observed alleles	Most frequent allele	Least frequent allele
INRA 63	11	358	147, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169	161 (0.36)	151, 155 (0.001)
BM 1818	**7**	373	249, 251, 255, 257, 259, 261, 263	255 (0.31)	261 (0.007)
CSRD 247	10	376	145, 219, 231, 233, 235, 237, 239, 241, 243, 245	243 (0.45)	231 (0.003)
MCM 527	13	375	140, 142, 148, 150, 152, 154, 158, 160, 162, 164, 166, 168, 170	154 (0.34)	142, 168 (0.003)
BM 1258	**16**	373	97, 99, 101, 103, 107, 109, 111, 113, 115, 117, 119, 121, 127, 129, 131, 137	101 (0.36)	107 (0.001)
OarFCB 48	13	348	149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 175	153 (0.37)	171 (0.001)
BM 7160	**7**	378	165, 167, 169, 173, 175, 177, 181	165 (0.42)	169 (0.001)
SRCRSP 8	8	378	213, 223, 225, 229, 233, 237, 241, 243	225 (0.42)	229 (0.005)
BM 1329	**7**	380	167, 169, 171, 173, 175, 177, 179	175 (0.40)	167 (0.001)
HSC	15	371	271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 301, 303, 305	**279 (0.28)**	277, 301, 305 (0.001)
SRCRSP 5	9	377	160, 162, 164, 168, 170, 172, 174, 176, 178	172 (0.39)	174 (0.001)
INRABERN 192	**7**	379	178, 186, 188, 190, 192, 198, 200	**188 (0.58)**	198 (0.001)
Average	10.25	372	-	-	-

k: Number of alleles per locus
N: Number of animals genotyped

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Only one (INRABERN 192) of the 12 markers had an allele with a frequency of above 0.5 (indicated in bold in Table 4.1), a factor that will have limited the strength of distinguishing power of the marker due to the high frequency of the allele in the population. The remaining markers in the panel demonstrated most frequent alleles of between 0.28 (HSC) and 0.45 (CSRD 247). The marker with the highest number of alleles per locus (k) was BM 1258 (16) and those with the lowest number of alleles per locus were BM 1818, BM 7160, BM 1329 and INRABERN 192 (all with 7). The mean number of alleles per locus for the marker panel in the population was 10.25, with a standard deviation of 3.31.

The observed and expected heterozygosity (H_O and H_E), Polymorphic Information Content (PIC), null allele frequency (F_{NULL}) and exclusion probability (PE) are presented in Table 4.2.

Table 4.2 Observed and expected heterozygosities, PIC values, F_{Null} and PE for 12 markers

Locus	H_O	H_E	PIC	F_{Null}	PE
INRA 63	0.696	0.772	0.738	+0.046	0.615
BM 1818	0.802	0.786	0.752	-0.010	0.596
CSRD 247	0.731	0.691	0.642	-0.028	0.721
MCM 527	0.696	0.760	0.723	+0.037	0.627
BM 1258	0.761	0.789	0.762	+0.016	0.572
OarFCB 48	0.822	0.761	0.725	-0.047	0.626
BM 7160	0.580	0.631	0.557	+0.041	0.788
SRCRSP 8	0.751	0.720	0.679	-0.021	0.679
BM 1329	0.732	0.709	0.660	-0.022	0.702
HSC	0.871	0.842	0.823	-0.019	0.477
SRCRSP 5	0.796	0.770	0.741	-0.019	0.605
INRABERN 192	0.699	0.629	0.602	-0.069	0.758
Average	0.745	0.738	0.700	-	0.647

H_O: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic Information content
F_{Null}: Null allele frequency
PE: Probability of exclusion for sire alone

Observed heterozygosity (H_O) ranged from 0.579 (BM 7160) to 0.871 (HSC), with a mean of 0.745. Expected heterozygosity (H_E) ranged from 0.629 (INRABERN 192) to 0.842 (HSC), with a mean of 0.738. The most informative markers in terms of Polymorphic Information Content (PIC) were HSC (0.823), BM1258 (0.762) and BM 1818 (0.752). The markers with the lowest Polymorphic Information

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Content (PIC) were BM 7160 (0.557), INRABERN 192 (0.602) and CSRD 247 (0.642). The markers with the highest null allele frequency were INRA 63 (+0.046) and BM 7160 (+0.041), however these are still lower than 0.05 and therefore are still suitable for use in parentage verification (Marshall *et al.,* 1998). Markers INRABERN 192 (-0.069) and OarFCB 48 (-0.047) showed the highest negative null allele frequency, an indication of an excess of heterozygotes in the population. Markers that performed with the highest probability of exclusion (PE), which is the ability of a marker to exclude a non-related sire as a potential sire (Marshall *et al.,* 1998), were BM 7160 (0.788), INRABERN 192 (0.758) and CSRD 247 (0.721), where those that performed at the lowest probability of exclusion were HSC (0.477), BM 1258 (0.572) and BM 1818 (0.596). The average probability of exclusion for all loci over the population was 0.647.

The markers included in the marker panel were further tested in order to determine whether the population was in Hardy-Weinberg equilibrium (Table 4.3).

Table 4.3 Results of the Hardy-Weinberg test for microsatellite markers

Microsatellite marker	df	Chi-Square	Probability	Significance
INRA 63	10	64.925	0.0000	***
BM 1818	10	5.070	0.8864	NS
CSRD 247	3	3.562	0.3128	NS
MCM 527	6	71.715	0.0000	***
BM 1258	6	22.410	0.0010	*
OarFCB 48	6	16.706	0.0104	NS
BM 7160	3	10.837	0.0126	NS
SRCRSP 8	3	4.747	0.1913	NS
BM 1329	6	22.292	0.0011	*
HSC	10	9.437	0.4912	NS
SRCRSP 5	10	4.812	0.9034	NS
INRABERN 192	3	30.855	0.0000	***

NS: not significant
*: 5% significance (P<0.05)
***: 0.1% significance (P<0.001)

Seven (BM 1818, CSRD 247, OarFCB 48, BM7160, SRCRSP 8, HSC and SRCRSP 5) of the 12 markers showed no significant deviation from Hardy-Weinberg Equilibrium, indicated by “NS” in table 4.3. A deviation from Hardy-Weinberg Equilibrium was observed for markers BM 1258 and BM 1329 at a 5%

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significance level, and for markers INRA 63, MCM 527 and INRABERN 192 at a 1% significance level, indicated by asterices in Table 4.3.

4.2 Parentage Analyses

A total of 381 individuals were genotyped using the verified marker panel. The population studied consisted of eight half-sib paternal families consisting of between 20 and 70 offspring each, a group of 40 unallocated offspring and a group of 16 putative sires. In all families only the paternal genotype was known.

Parentage analysis was carried out including all families, unallocated offspring and putative sires as a combined single population. The analysis process was the same for all of the families and results are presented in a similar way. The results of Families D and E were included in this chapter (Table 4.4 and 4.5) and the remaining families (Family A, B, C, F, G, H and Group sires) were attached as Addendum A. The first column of each table contains the on-farm recorded identification number for each offspring, the second column contains the identification number of the sire that was assigned to each offspring by Cervus™ in this study, the third column contains the identification number of the sire that was recorded for each offspring by the breeder, the fourth column contains the number of loci that were compared between the allocated sire and the offspring by Cervus™, the fifth column contains the number of loci that did not correspond between the offspring and allocated sire, the sixth column contains the LOD score for each parent-offspring allocation and the seventh column contains the confidence with which the allocation is scored by Cervus™.

The original size of family D as recorded by the breeder was 35 offspring. The family size increased to 37 offspring after the DNA-based parentage analysis. Cervus allocated an additional nine offspring to the family while reallocating seven offspring from the original family D to new sires. 28 of the original 35 offspring in family D were verified by the DNA-based parentage analysis to have been sired by sire D1. The kids that were newly allocated to family D by Cervus have their previously recorded sires printed in bold in Table 4.4.

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Table 4.4 Parentage allocation of family D with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/08208	D1	D1	12	0	4.990	*
142/08237	D1	D1	12	0	4.430	*
142/08241	D1	D1	10	0	3.930	*
142/08242	D1	D1	12	0	7.420	*
142/08243	D1	D1	12	0	8.970	*
142/08244	D1	D1	11	0	6.060	*
142/08253	D1	D1	12	0	5.400	*
142/08262	D1	D1	12	0	3.920	*
142/08271	D1	D1	12	0	6.070	*
142/08283	D1	D1	12	1	0.590	*
142/08298	D1	D1	12	0	5.840	*
142/08316	D1	D1	12	0	5.980	*
142/08317	D1	D1	12	0	6.960	*
142/08318	D1	D1	12	0	6.580	*
142/08339	D1	D1	12	0	1.800	*
142/08356	D1	D1	12	0	4.680	*
142/08360	D1	D1	12	1	3.410	*
215/08706	D1	D1	10	0	5.230	*
215/08707	D1	D1	12	0	2.530	*
215/08708	D1	D1	12	1	1.720	*
215/08742	D1	D1	10	0	6.630	*
215/08774	D1	D1	12	0	6.430	*
215/08775	D1	D1	12	1	4.090	*
215/08789	D1	D1	11	0	4.970	*
215/08792	D1	D1	12	0	4.480	*
317/08049	D1	D1	8	0	2.580	*
317/08081	D1	D1	12	2	-3.880	*
317/08082	D1	D1	12	0	6.190	*
142/06238[ac]	D1	**E1**	12	0	10.900	*
142/06298[ac]	D1	**E1**	11	0	3.070	*
142/06309[ac]	D1	**E1**	12	1	0.873	*
142/06310[ac]	D1	**E1**	12	0	5.050	*
154/06618[ac]	D1	**E1**	12	1	3.440	*
317/06065[ac]	D1	**E1**	12	1	3.020	*
154/06605[ac]	D1	**D1**	12	2	-6.380	*
142/08369[b]	S1	**D1**	12	2	0.979	*
215/08753[b]	S2	**D1**	11	3	-4.630	*
317/08046[b]	S4	**D1**	12	1	1.890	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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In instances where the breeder-recorded sire and the DNA-based allocated sire were related, the Cervus results were verified by cross-referencing with on-farm records. On consulting the kids' and sires' birth dates it was found that sire D1 was not old enough to have sired these kids. Angora goat bucks enter the breeding herd for the first time at 18 months of age (personal communication – Dr M Snyman, GADI, South African National Department of Agriculture in the Eastern Cape), therefore, the sire would have to be at least two years of age at the kidding date of his offspring. Kids indicated by superscript "c" (Table 4.4) are offspring born in 2006, the same year of birth of the sire allocated by the DNA-based parentage verification, D1. In these cases, the kids were reassigned to the original sire that was recorded by the breeder, E1, who is the father of the DNA-based allocated sire, D1. These kids (indicated by superscript "a" in Table 4.4 were removed from family D and allocated to family E, of sire E1).

Parentage allocations were checked further by manually comparing the individual genotypes at each locus. Each marker genotype of the offspring was compared with both sires to identify any mismatches. Offspring 142/08369 had two mismatches with the DNA-based allocated "new" sire (group sire S1, printed in bold in Table 4.4) at loci INRA63 and BM7160 and only one mismatch with the breeder-allocated "old" sire (D1). The LOD score for the allocation to group sire S1 was 0.979, which falls within the "ambiguous" parentage range (between negative three and positive three). This indicates a low statistical confidence in the DNA-based parentage assignment of the kid to group sire S1. The kid was, therefore, assigned to the breeder-recorded sire (D1) on account of one locus mismatch in contrast with the mismatches at two loci with the DNA-based allocated sire (group sire S1), as well as a low LOD score for the allocation by Cervus.

After taking the LOD score into account, where there were an equal number of mismatches between each sire and the offspring, birth dates of the kids were consulted. Kids born from September onwards were assigned to the DNA-based allocated sire and kids born before September were assigned to the breeder-recorded sire (as discussed in CHAPTER 3: Materials and Methods). Offspring 317/08046 was allocated to group sire S4 with a LOD score of 1.890. There was also a mismatch with both sires at locus MCM527. The kid was born in mid-August and, therefore was assigned to the sire recorded by the breeder (sire D1). Offspring 215/08753 had two mismatches with both the DNA allocated (S2) and breeder-recorded (D1) sires and was reassigned to the breeder-recorded sire (D1) based on the LOD score of -4.63 for the DNA allocation. The two kids (142/08369 and 317/08046) were reallocated to family D after Cervus allocated them to two different group sires (printed in bold in Table 4.4). The offspring are indicated by superscript "b" in Table 4.4. These two offspring were subsequently added to family D

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(indicated by superscript "c"), thereby increasing the number of offspring that were assigned to family D from the originally recorded family to 30 kids.

Table 4.5 Parentage allocation of family E with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/06229	E1	E1	12	0	7.570	*
142/06233	E1	E1	12	0	4.860	*
142/06272	E1	E1	12	0	7.660	*
142/06280	E1	E1	12	1	2.880	*
142/06282	E1	E1	12	0	9.310	*
142/06283	E1	E1	10	0	6.760	*
142/06290	E1	E1	12	0	5.380	*
142/06302	E1	E1	12	1	3.160	*
142/06306	E1	E1	12	0	7.950	*
142/06308	E1	E1	12	2	-4.370	*
142/06316	E1	E1	11	0	3.780	*
154/06606	E1	E1	12	0	6.140	*
154/06610	E1	E1	11	0	8.310	*
154/06611	E1	E1	12	1	3.280	*
317/06020	E1	E1	12	1	1.930	*
317/06024	E1	E1	12	0	6.860	*
317/06025	E1	E1	12	0	5.530	*
317/06053	E1	E1	10	0	6.320	*
317/06054	E1	E1	12	1	4.190	*
317/06067	E1	E1	12	1	2.900	*
317/06071	E1	E1	12	0	6.190	*
317/06074	E1	E1	11	0	8.020	*
317/06084	E1	E1	11	0	5.160	*
154/06661	E1	**G1**	12	0	2.550	*
317/06058	E1	**G1**	12	0	6.180	*
142/06238[b]	D1	E1	12	0	10.900	*
142/06298[b]	D1	E1	11	0	3.070	*
142/06309[b]	D1	E1	12	1	0.873	*
142/06310[b]	D1	E1	12	0	5.050	*
154/06618[b]	D1	E1	12	1	3.440	*
317/06065[b]	D1	E1	12	1	3.020	*
142/06301[bc]	S13	E1	12	2	0.632	*
142/06234[bc]	S15	E1	12	1	1.530	*
142/06260[bc]	S15	E1	10	0	5.280	*
215/06815[bc]	S15	E1	12	1	4.540	*
215/06824[bc]	S15	E1	12	1	2.880	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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The parentage analysis for family E is presented in Table 4.5. The original size of the family as recorded by the breeder was 35 offspring. Two offspring were assigned to family E by Cervus that were previously recorded by the breeder as being part of family G. The previously recorded sire of these two offspring are printed in bold in Table 4.5. 23 of the original 25 offspring were verified by Cervus to have been sired by sire E1.

Five offspring were assigned by Cervus to group sires that were less than two years older than them and therefore were reassigned to family E, as they were recorded to have belonged to by the breeder and are indicated by superscript "bc" in Table 4.5. Offspring 142/06301 was born in 2006 and was allocated to sire S13 by Cervus, a buck born in 2005. Offspring 142/06234, 142/06260, 215/06815 and 215/06824 were all born in 2006 and were allocated to sire S15, born in the same year.

Six additional offspring (142/06238, 142/06298, 142/06309, 142/06310, 154/06618 and 317/06065) were removed from family D because sire D1 was less than two years younger than them, as discussed previously, were added to family E and are indicated by superscript "b" (Table 4.5). No offspring were removed from family E after the DNA-based analysis. Family E ended with a final number of 36 offspring. The final family sizes as well as number of offspring added to or removed from each family is presented in Table 4.6.

Table 4.6 Change in number of offspring per half-sib family by the DNA-based parentage allocation

Family	Sire	Original family size	Number of offspring added	Number of offspring removed	Final family size
A	A1	26	5	2	29
B	B1	70	5	12	63
C	C1	34	3	3	34
D	D1	35	0	5	30
E	E1	35	2	1	36
F	F1	25	6	1	30
G	G1	70	7	5	72
H	H1	25	0	3	22

The number of kids allocated to each group (putative) sire are shown in Table 4.7. None of these sires' previously recorded offspring were taken into account in the parentage analysis.

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Table 4.7 Number of offspring allocated to the 16 putative group sires based on the DNA-verified parentage allocation

Sire	Offspring allocated
S1	1
S2	2
S3	1
S4	3
S5	10
S6	2
S7	1
S8	3
S9	0
S10	0
S11	4
S12	7
S13	1
S14	2
S15	3
S16	1

Sire S5 received the most offspring (10 kids) of all the group sires, as shown in Table 4.7. Sires S9 and S10 were allocated no offspring and S1, S3, S7, S13 and S16 were allocated one kid each during the DNA-based parentage analysis.

An overall summary of the parentage analysis is outlined in Table 4.8. This is a representation of the quantified error and incompleteness of the breeder's recorded pedigrees. There were 357 offspring in total included in the analysis. Cervus could allocate all offspring to a sire from the DNA results. 11 of the breeder's pedigree records (AI and single-sire mating records) were found incorrect by the DNA-based verification, this amounted to 3.1% of the pedigree). 40 offspring did not have recorded paternity, and all 40 were successfully allocated new sires by the DNA-based parentage allocation; this constituted 11.2% of the whole pedigree. Finally, the overall measure of pedigree incompleteness, consisting of both errors and missing records, amounted to 51 records and 14.3% of the entire pedigree as presented in Table 4.8.

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Table 4.8 Summary of overall parentage analysis results

	Number	Percentage of the entire pedigree
Total offspring	357	100%
Total number of parentage assignments by Cervus	357	100%
Successful assignments	314	88%
Inconclusive assignments	44	12.3%
Incorrect on-farm paternity records	11	3.1%
New parentage assignments	40	11.2%
Pedigree records amended	51	14.3%

4.3 Estimated Breeding Values (EBVs) and ranking of sires

In order to quantify the impact of pedigree integrity on selection accuracy and genetic progress, Estimated Breeding Values (EBVs) of each of the sires were calculated, firstly, using the pedigrees received from the breeder and then, secondly, by comparing with the EBVs calculated using the DNA-verified pedigrees. The EBVs were calculated using Best Linear Unbiased Prediction (BLUP) as back solutions when fitting single-trait animal models using the ASREML programme of Gilmour *et al*. (2009).

Economically important traits included were birth weight (W0 in kg), weaning weight (W4 in kg) and body weight at 8, 12 and 16 months of age (W8, W12 and W16 in kg), fleece weight (FW in kg) and fibre diameter (FD in µm) recorded at the second shearing. Birth weight, weaning weight and 8-month body weights were recorded in both male and female kids, while 12- and 16-month body weights were only recorded for the female kids.

The BLUP results for EBV estimations of fleece traits are given in Table 4.9. There are two values presented for each trait: firstly, from calculations resulting from analysis of the breeder-recorded pedigrees and, secondly, from the DNA-verified pedigrees, these are represented by superscript “1” and “2” respectively in the headings in Table 4.9.The three highest and three lowest rankings are printed in bold in Tables 4.9, 4.10 and 4.11. The accuracies of each EBV are printed as superscripts of each EBV value. Group sire S2 had no kids assigned to him by the breeder, precluding the calculation of his EBVs according to the breeder-recorded pedigree, and was therefore omitted from the EBV analysis. No offspring were assigned to group sires S9 and S10 and, therefore they were also excluded from this section.

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Table 4.9 Estimated Breeding Values, Estimated Breeding Value accuracies (superscripts) and rankings of 21 sires for fleece traits (fleece weight and fibre diameter) according to pedigrees recorded by the breeder and DNA-verified pedigrees

Sire	FW[1]	FW[2]	FW[1] Rank	FW[2] Rank	FD[1]	FD[2]	FD[1] Rank	FD[2] Rank
A1	0.029^{88}	0.039^{89}	10	10	2.51^{96}	2.07^{94}	**19**	**20**
B1	**0.197^{90}**	0.174^{89}	**3**	5	1.97^{97}	1.51^{95}	17	16
C1	-0.030^{82}	-0.007^{83}	18	18	0.53^{95}	0.54^{90}	9	10
D1	**0.224^{79}**	**0.217^{77}**	**1**	**3**	-1.27^{93}	-1.12^{86}	**1**	**1**
E1	0.172^{49}	**0.311^{86}**	4	**1**	0.28^{56}	0.49^{93}	7	9
F1	**-0.036^{44}**	**-0.077^{77}**	**20**	**21**	0.15^{57}	1.03^{87}	4	13
G1	0.107^{58}	0.135^{89}	8	8	1.88^{80}	1.85^{94}	16	18
H1	-0.020^{79}	-0.001^{77}	17	17	1.33^{94}	1.46^{88}	11	15
S1	0.075^{49}	0.088^{58}	9	9	3.17^{77}	-0.09^{74}	**21**	**3**
S3	0.006^{44}	0.028^{43}	14	13	-0.42^{63}	-0.06^{56}	**2**	4
S4	0.014^{49}	0.009^{54}	13	16	1.69^{55}	0.37^{61}	15	8
S5	**0.199^{62}**	0.192^{58}	**2**	4	2.44^{82}	1.59^{73}	18	17
S6	0.159^{54}	0.136^{58}	5	7	1.01^{78}	0.55^{69}	10	11
S7	**-0.050^{49}**	**-0.023^{49}**	**21**	**19**	1.46^{57}	1.30^{56}	13	14
S8	**-0.033^{85}**	**-0.026^{83}**	**19**	**20**	0.47^{96}	0.23^{92}	8	7
S11	0.003^{49}	0.038^{74}	15	11	0.26^{77}	-0.43^{85}	6	**2**
S12	0.028^{49}	0.034^{54}	11	12	0.25^{78}	0.00^{68}	5	5
S13	0.018^{49}	0.021^{49}	12	15	1.51^{54}	1.02^{55}	14	12
S14	-0.007^{54}	0.027^{54}	16	14	0.03^{78}	0.10^{69}	**3**	6
S15	0.113^{62}	**0.264^{63}**	7	**2**	1.46^{82}	4.86^{74}	12	**21**
S16	0.129^{58}	0.144^{58}	6	6	2.68^{80}	1.93^{69}	**20**	**19**

[1]: Value calculated using the breeder-recorded pedigrees
[2]: Value calculated using the DNA-verified pedigrees
FW: Fleece weight EBV
FW Rank: Sire rank for fleece weight EBV
FD: Fibre diameter
FD Rank: Sire rank for fibre diameter
Accuracies of Estimated Breeding Values are printed as superscripts

From the BLUP analysis for fleece traits, distinct differences can be seen in the EBV estimation and sire ranking, and, to a smaller extent, EBV accuracies. For fleece weight, the three highest ranking sires in the breeder-recorded pedigree (D1, S5 and B1) differed from those in the DNA-verified pedigree (E1, S15 and D1); the three lowest ranking sires (F1, S7 and S8) remained the same, even though the order changed slightly. Six sires (S1, A1, C1, H1, G1 and S16) retained the same rank in the DNA-verified pedigree as in the breeder-recorded pedigree. EBV accuracies showed an overall improvement from the breeder-recorded pedigree to the DNA-verified pedigree, with the greatest increase seen in sires F1 (lower ranking sire), from 44% to 77%, E1 from 49% to 86% and S11 from 49% to 74% accuracy. The three sires with the highest accuracy of EBV estimation were those with the most offspring.

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The results for fibre diameter differed from those of fleece weight, which is what can be expected as the two are positively (but unfavourably) correlated (Pattie *et al*., 1990). The highest ranking sire (D1) remained the same from the breeder-recorded pedigree to the DNA-verified pedigree. The other two highest ranking sires differed between pedigrees. The second and third ranking sire in the breeder-recorded pedigree were S3 and S14 respectively, and S11 and S1 respectively for the DNA-verified pedigree. Two of the three lowest sires corresponded between the two pedigrees, where the third of the lowest ranking sires, sire (S1), that was ranked the lowest in the breeder-recorded pedigree was ranked the third highest in the DNA-verified pedigree. There was an erratic response in the changes in the accuracy of the EBVs in the two pedigrees. Sire S3 had a low EBV for fibre diameter accuracy on account of the fact that only one kid was allocated to him. The accuracies of the calculation of the EBVs for fibre diameter are generally higher than those of fleece weight, with the three animals with the highest accuracies remaining the same for both traits.

The results from the BLUP analysis of early body weight traits are presented in Table 4.10. The traits included are birth weight (W0), Weaning weight (W4), which was measured at four months of age, and eight month weight (W8), along with the respective EBV accuracies and sire rankings. For birth weight (W0), one sire (S8) remained in the top three ranked sires after the DNA-based pedigrees were arranged. Sire D1 was ranked the lowest performing for birth weight in the pedigrees recorded by the breeder but ranked second in the DNA-verified pedigree. Further, there was a significant change in ranks from the on-farm pedigree to the DNA-verified pedigree. The sire (B1) that was ranked second highest in the breeder-recorded pedigree was ranked at 18th position out of 21 sires in the DNA-verified pedigrees. A general trend can be seen in the changes in accuracies between pedigrees when looking at all three early body weights. The largest improvement in EBV accuracy of all three traits was observed in sires S1, F1, E1, G1 and S11. Furthermore, the sires (A1, B1 and C1) with the highest EBV accuracy in all three traits corresponded with those with the highest accuracies of the EBVs of fleece traits. The sire rankings for eight month weight showed a similar trend to weaning weight where the order of sires was significantly shuffled. Sire C1 remained in third ranked position (Table 4.10) in the DNA-verified pedigree after the parentage allocation, showing a similar response to the pedigree amendments as with weaning weight. Additionally, the other top ranking sires (S3 and S8) from the breeder-recorded pedigree moved to the lowest positions in the DNA-verified pedigree. Similarly, the sires that ranked lowest in the breeder-recorded pedigree increased considerably in rank after the DNA-based parentage allocation. Corresponding trends were observed in all early body weight traits. The same three animals (A1, B1 and C1) had EBVs with the highest accuracy which was consistent with both birth weight and weaning weight

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Table 4.10 EBVs, EBV accuracies and sire rankings for body weights (birth weight, weaning weight and eight month weight) according to pedigrees recorded by the breeder and DNA-verified pedigrees.

Sire	W0[1]	W0[2]	W0[1] Rank	WO[2] Rank	W4[1]	W4[2]	W4[1] Rank	W4[2] Rank	W8[1]	W8[2]	W8[1] Rank	W8[2] Rank
A1	-0.068^{94}	0.051^{94}	**20**	13	-0.80^{92}	1.72^{91}	**21**	**1**	-0.50^{86}	1.12^{87}	**20**	4
B1	0.114^{94}	0.011^{94}	**2**	18	0.66^{93}	0.33^{92}	5	15	0.53^{88}	0.02^{88}	6	17
C1	0.088^{92}	0.045^{92}	4	14	1.63^{87}	1.45^{86}	**2**	**3**	0.91^{79}	1.49^{79}	**3**	**3**
D1	-0.116^{82}	0.145^{81}	**21**	**2**	0.29^{76}	1.67^{74}	9	**2**	-0.03^{73}	1.85^{72}	14	**1**
E1	-0.012^{54}	0.111^{94}	15	5	-0.24^{47}	0.87^{89}	18	9	-0.31^{45}	0.63^{84}	**19**	7
F1	0.037^{58}	0.027^{88}	7	16	0.13^{53}	0.43^{82}	13	14	0.10^{47}	0.43^{71}	12	10
G1	-0.029^{61}	0.071^{92}	18	7	-0.77^{57}	1.35^{87}	**20**	4	-0.86^{54}	0.21^{84}	**21**	15
H1	0.035^{88}	0.145^{88}	9	**3**	-0.38^{83}	0.86^{81}	**19**	10	-0.22^{74}	0.53^{73}	18	9
S1	0.030^{51}	0.005^{78}	10	**19**	0.79^{44}	-1.19^{71}	4	**21**	0.41^{42}	-0.23^{63}	9	**21**
S3	-0.002^{79}	0.070^{78}	13	8	2.00^{78}	0.15^{76}	**1**	18	1.57^{67}	0.02^{68}	**1**	18
S4	-0.004^{51}	0.032^{55}	14	15	-0.22^{52}	0.92^{56}	17	6	-0.10^{44}	0.54^{49}	16	8
S5	0.051^{61}	-0.054^{65}	6	**21**	0.52^{57}	0.30^{58}	8	16	0.85^{54}	0.40^{56}	4	11
S6	0.018^{58}	-0.042^{62}	12	**20**	0.62^{54}	-0.10^{56}	7	**19**	0.53^{50}	0.21^{53}	5	14
S7	0.022^{58}	0.103^{59}	11	6	-0.08^{54}	1.09^{54}	15	5	0.48^{48}	1.03^{50}	8	5
S8	0.101^{88}	0.154^{88}	**3**	**1**	0.94^{84}	0.51^{83}	**3**	13	1.01^{79}	-0.05^{79}	**2**	**19**
S11	-0.040^{51}	0.143^{81}	**19**	4	0.16^{45}	-0.71^{73}	12	**20**	-0.16^{30}	0.07^{61}	17	16
S12	0.037^{47}	0.066^{59}	8	11	0.63^{41}	0.18^{50}	6	17	0.31^{37}	0.27^{46}	10	12
S13	0.062^{61}	0.070^{62}	5	9	-0.16^{56}	0.72^{57}	16	12	-0.08^{53}	-0.08^{54}	15	**20**
S14	-0.014^{77}	0.020^{76}	16	17	0.18^{70}	0.90^{69}	10	7	0.23^{51}	1.60^{53}	11	**2**
S15	-0.016^{64}	0.060^{68}	17	12	0.17^{60}	0.81^{61}	11	11	-0.01^{56}	0.89^{59}	13	6
S16	0.133^{61}	0.069^{62}	**1**	10	0.10^{56}	0.88^{56}	14	8	0.51^{53}	0.26^{54}	7	13

[1]: Value calculated using the breeder-recorded pedigrees
[2]: Value calculated using the DNA-verified pedigrees
W0: Birth weight EBV
W0 Rank: Sire rank for birth weight EBV
W4: Weaning weight EBV
W4 Rank: Sire rank for weaning weight EBV
W8: Eight month body weight EBV
W8 Rank: Sire rank for eight month body weight EBV
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The EBVs, their accuracies as well as sire rankings for mature body weight traits, namely yearling weight (W12) and 16 month weight (W16), are presented in Table 4.11. The change in EBVs and sire ranking in yearling weight showed a marked difference from that seen in the early body weights, as presented in Table 4.11 and Table 4.10 respectively. The first and second ranking sires (C1 and A1 respectively) remained the same after the DNA-based parentage allocation. The third top ranking sire (S6) in the breeder-recorded pedigree decreased to 14th position after the DNA-based parentage allocation. The lowest ranking sires (F1, H1 and S8) in the breeder-recorded pedigree increased in ranking up to the top ten performing sires in the DNA-verified pedigree. Similarly, all three lowest ranking sires in the DNA-verified pedigree were originally ranked in the top ten of the breeder-recorded pedigree. The greatest improvement in accuracy in the DNA-verified pedigree was seen in sire E1, with a 48% increase.

Table 4.11 EBVs, EBV accuracies and sire rankings for mature body weights (yearling weight and 16 month weight) according to pedigrees recorded by the breeder and DNA-verified pedigrees.

Sire	W12[1]	W12[2]	W12[1] Rank	W12[2] Rank	W16[1]	W16[2]	W16[1] Rank	W16[2] Rank
A1	0.57^{83}	1.48^{92}	**2**	**2**	-1.42^{52}	1.48^{89}	18	5
B1	0.12^{88}	0.83^{94}	8	6	-0.52^{48}	-0.25^{91}	14	18
C1	0.63^{77}	1.94^{88}	**1**	**1**	0.11^{56}	1.89^{83}	9	**3**
D1	0.04^{71}	-0.20^{82}	13	16	2.58^{59}	0.80^{81}	**2**	9
E1	-0.05^{41}	0.07^{89}	17	12	-2.28^{53}	-0.31^{89}	**20**	**19**
F1	-0.22^{41}	0.43^{82}	**21**	8	-2.16^{58}	1.57^{84}	**19**	4
G1	0.04^{47}	-0.26^{88}	12	18	0.70^{61}	-0.44^{88}	5	**20**
H1	-0.07^{74}	1.34^{86}	**20**	4	0.47^{56}	0.21^{73}	6	14
S1	0.20^{42}	-0.99^{76}	6	**21**	-0.15^{32}	0.23^{46}	10	13
S3	0.06^{63}	-0.50^{78}	11	**20**	0.19^{43}	0.33^{77}	8	12
S4	0.24^{42}	-0.40^{58}	5	**19**	-0.50^{50}	0.36^{57}	13	11
S5	0.31^{51}	0.09^{65}	4	11	-1.05^{37}	-0.16^{58}	16	17
S6	0.38^{51}	-0.02^{63}	**3**	14	-0.17^{46}	-0.67^{52}	11	**21**
S7	0.01^{41}	0.86^{55}	14	5	-0.64^{59}	1.95^{56}	15	**2**
S8	-0.06^{76}	0.68^{87}	**19**	7	3.28^{74}	1.13^{75}	**1**	6
S11	-0.06^{11}	-0.16^{55}	18	15	-3.67^{71}	-0.13^{81}	**21**	16
S12	0.08^{39}	-0.00^{65}	9	13	1.47^{37}	0.49^{56}	4	10
S13	0.18^{47}	-0.24^{58}	7	17	-1.31^{59}	0.01^{57}	17	15
S14	0.06^{61}	1.36^{77}	10	**3**	-0.36^{55}	2.35^{66}	12	**1**
S15	0.00^{49}	0.16^{65}	15	9	0.42^{52}	0.81^{67}	7	8
S16	-0.01^{45}	0.11^{57}	16	10	2.01^{60}	0.95^{57}	**3**	7

[1]: Value calculated using the breeder-recorded pedigrees
[2]: Value calculated using the DNA-verified pedigrees
W12: Yearling weight EBV
W12 Rank: Sire rank for yearling weight EBV
W16: 16 month weight EBV
W16 Rank: Sire rank for 16 month weight
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The final trait included in the EBV analyses was 16 month mature weight (W16), as per Table 4.11. There was no correlation between the three top ranking sires in the breeder-recorded pedigree and the DNA-verified pedigree, the ranking order shuffled considerably. The top three sires (S8, D1 and S16 respectively) in the breeder-recorded pedigree all decreased in position after the DNA-based parentage allocation, but all remained in the top ten ranks. One of the bottom ranking sires (F1) from the breeder-recorded pedigree increased significantly in rank up to 4th position in the DNA-verified pedigree. The three sires (S14, S7 and C1) that were the three highest performing in the DNA-pedigree (respectively) were originally placed in 12th, 15th and ninth position respectively in the breeder-recorded pedigree. Sire G1, ranked very low at 20th in the DNA-verified pedigree was originally placed at 5th in the EBV rankings in the breeder-recorded pedigree. The greatest improvement in EBV accuracy was seen in sire B1, with a 43% increase.

Overall, verification of parentage using DNA technology has a distinct impact on sire ranking and the accuracies of the EBV estimations. The sire that performed consistently well in the fleece weights and early body weights was D1, being ranked in the top three for all five traits, as presented in Tables 4.9 and 4.10.

The results of the study verify the suitability of the microsatellite marker panel designed by Friedrich (2009) for parentage verification in South African Angora goats and bear witness to the impact that even slight inaccuracies in pedigree records can have on selection decisions made by breeders.

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CHAPTER 5: Discussion

Current trends in livestock breeding worldwide demonstrate the advantage of selecting animals based on more than animal phenotypes and on-farm records. Studies in animal genomics have presented the opportunity to incorporate genetic information into breeding programmes (Womack, 2005). Pedigree inaccuracies, particularly paternity errors, have substantial repercussions on national genetic evaluations, genetic trends and genetic advancement (Banos *et al.,* 2001; Visscher *et al.,* 2002). The value of DNA-based parentage testing for the South African Angora goat industry was investigated with reference to the Expected Breeding Values (EBVs) routinely estimated at GADI. Currently there are around 800 Angora goat breeders in South Africa. No producers are currently taking part in the National Small Stock Improvement Scheme (although there are a few breeders that participate in recording projects managed by GADI) or doing routine DNA marker-based parentage testing (personal communication: Dr M.A. Snyman, GADI, Middelburg, National Department of Agriculture, Eastern Cape).

5.1 Parentage assignment

The importance of pedigree soundness for genetic progress has been demonstrated by several studies in a number of livestock species (Ron *et al.,* 1996; Israel & Weller, 2000; Banos *et al.,* 2001; Visscher *et al.,* 2002; Senneke *et al.,* 2004; Gomez-Raya *et al.,* 2008), however unsound pedigrees remain a recurring limitation to genetic progress in many livestock breeding systems. Angora goat breeding in South Africa stands to benefit greatly by the advantage offered by DNA marker-based parentage verification in maintaining its competitive position in the global mohair market. A previous study by Visser *et al.* (2011) established that the error in South African Angora goat pedigrees was approximately 25%. Parentage relationships are assumed to be correctly recorded when breeders make selection decisions based on phenotypic performance.

Paternity assignment in the current study was done using a panel of 12 microsatellite markers on a population of 381 South African Angora goats. The current study employed a larger population than studies on goats by Visser *et al.* (2011) and De Araújo *et al.* (2010) and smaller than the population of Bolormaa *et al.* (2008) and Jimenez-Gamero *et al.* (2006). Larger populations require more microsatellite markers for acceptable accuracy in analyses as there are more candidate parents (Liu & Cordes, 2004). Parentage was verified in eight half-sib families (as recorded by the breeder), and 40 previously unallocated offspring were allocated new sires. 16 putative sires were also included as candidate parents. These animals were included in the overmating sire herd at the end of the mating season. The purpose of using animals in pre-recorded half-sib families was to quantify the error in the breeder's on-farm pedigree records and rectify the mistakes.

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Genotypic results for the parentage analysis were analysed using Cervus™ 3.0, a software chosen based on its ability to account for genotyping errors (Marshall *et al.,* 1998). There were no cases where Cervus could not allocate a sire to an offspring based on maximum likelihood. There were, however, a few matches that were inconclusive owing to the relatedness of the sires in the population. There were a number of father-son relationships within the sire group and, therefore, as a result of sharing common alleles, some offspring were erroneously allocated to the other related sire. In such cases (as discussed in CHAPTER 4: RESULTS) the birth date of the kid was consulted and, based on their birth date were allocated to either the on-farm recorded sire or the DNA-verified sire. The study by Fisher *et al.* (2009) illustrates the higher confidence achieved when combining DNA marker-based parentage allocation with on-farm records. In Fisher's (2009) study the marker panel was only able to successfully allocate 75% of the offspring to the correct sire and dam without the use of on-farm records, in contrast to the present study, where 88% successful paternal allocation rate was achieved. Another option for populations that are suspected of being inbred is to include more markers in the panel, as described by Altet *et al.* (2001), for higher resolution power.

The breeder of the animals used in the study made use of AI, single sire mating and group mating (or overmating). The greatest degree of error in on-farm recording is expected to originate from does that did not fall pregnant during the AI or single-sire mating periods and were successfully impregnated during the overmating period at the end of the breeding season. Therefore, kids born later in the season were most likely conceived during the overmating period and, therefore have questionable paternity.

The current study detected 3.1% errors (11 animals) in the on-farm pedigree records, which shows that human recording errors during AI and single-sire mating was minimal. The total records amended included 40 offspring that did not have on-farm paternity records and amounted to 14.3% (51 animals) of all the offspring in the population. This value is lower than what was previously found by Visser *et al.* (2011), probably as a result of the breeding practices on this particular farm and possibly the larger population size of the current study. Higher error rates would be expected in production systems where only natural mating is used as bucks are known to jump fences. The incorrect and incomplete pedigree records found in the current study were of a larger proportion than found by Visscher *et al.* (2002), Rehout *et al.* (2006) and Van Eenennaam *et al.* (2007) in cattle, and smaller in proportion than found by Dodds *et al.* (2007) in sheep as reported in Table 5.1.

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Table 5.1 Error rate in on-farm parentage records of different studies

Study	Species	Incomplete or inaccurate pedigrees
Current	Goats	14.3%
Visser *et al.* (2011)	Goats	25%
Van Eenennaam *et al.* (2007)	Cattle	12%
Rehout *et al.* (2006)	Cattle	12%
Visscher *et al.* (2002)	Cattle	10%
Dodds *et al.* (2007)	Sheep	15%

As mentioned in previous chapters, parentage can be assigned based on statistical confidence parameters including the LOD score. In a number of cases, offspring were assigned a sire with a high statistical confidence but with a low LOD score. A reason for this could be that there were mismatches at one or more loci between sire and offspring but the confidence was still high as parentage is allocated based on (among other parameters) allele frequencies at the different loci (Marshall, *et al.,*1998). Statistical confidence is based on more than just the number of matches or mismatches between sire and offspring. Only where there was strong conflicting evidence regarding an allocation (such as a sire being born in the same year as his allocated offspring) was a Cervus allocation overruled. This was discussed at length in CHAPTER 4: RESULTS and was a result of a number of sires in the breeding herd being related and sharing common alleles. On the whole, LOD scores from the parentage analysis were mostly positive and all allocations were of high statistical confidence.

Kids may be erroneously allocated to the wrong sire for a number of reasons, such as missing parental genotypes, null alleles, genotyping errors and relatedness among candidate parents. In the current study, all potential sires that were present in the breeding herd on the farm were included in the study population and, therefore, no parental genotypes were missing.

Null alleles pose complications in parentage studies. The review by Dakin & Avise (2004) stated that the presence of null alleles in a large enough population (more than 100 individuals) did not have an effect on the PE of a microsatellite panel when the null allele frequency for each marker was below 0.2. Fortunately in the current study, no markers showed a null allele frequency higher than +0.046. The complication occurs, however, when a true parent may be falsely excluded when a kid is heterozygous for the null allele (Dakin & Avise, 2004). In the current study, on-farm data (kid birth dates) was used to verify the parentage allocated by Cervus in problem cases, therefore the possible presence of null alleles

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in one or more of the loci did not compromise the allocations. Dakin & Avise (2004) advise the use of multiple loci for comparison in order to compensate for null alleles as other corrective measures (such as redesigning primers for the affected loci) were often ineffective and expensive.

Genotyping errors can obscure the scoring of alleles. Pompanon *et al.* (2005) illustrate how genotyping errors arise and how they affect allele scoring. There are two types of genotyping errors: mis-scoring, which is the scoring of an inanimate peak or stutter pattern as an allele, and allelic dropout, where an allele is not scored but appears as a stutter band (Pompanon *et al.,* 2005). This can result in an allele being scored wrong or not being scored at all, and offspring will not show any matches to the true sire's genotype. According to Hoffman & Amos (2005), genotyping errors can arise as a result of poor quality DNA. If the template is of poor condition the amplification of alleles is likely to be sub-optimal. Errors may appear in good quality DNA by a mutation in the primer binding site causing DNA polymerase slippage making it difficult to score some alleles correctly (Hoffman & Amos, 2005). High DNA quality was used in the current study with a concentration of between 60 and 120 ng/µL. Researchers are cautioned in the use of highly polymorphic loci as Hoffman & Amos (2005) found that loci with a greater number of alleles per locus showed larger stutter bands and were more prone to genotyping errors, and even 1% genotyping error frequency could cause up to 20% of paternities being unresolved (Marshall *et al.*, 1998). The present study did not reflect any major effects of genotyping errors. In individual cases where alleles at a particular locus may have been mis-scored, the alleles were scrutinised and manually edited while taking genotypes across all loci into account.

It was found in the present study that relatedness among the breeding parents in a herd can have a negative effect on parentage tests. Related individuals (especially sibling and parent-offspring pairs) tend to have alleles in common. This could result in offspring being allocated to another sire that is related to the true sire when using maximum likelihood allocation methods. Sherman *et al.* (2004) carried out a study to quantify the effect of sire relatedness and number of candidate sires on the robustness of a parentage assay. They state that the relatedness and size of the candidate parent population has a direct effect on the probability of assigning unambiguous parentage, and found that up to 15% of the offspring were not assigned unambiguous parentage as a result of half-sibs in the multiple sire breeding herd. Similar results were realised in the present study where 12.3% of the offspring were allocated to a wrong sire that was related to the true sire. The sire breeding herd in the present study contained a number of offspring-sire pairs. This is probably a result of the breeder keeping his own male offspring as breeding stock. The negative effects of this are likely to be similar to having half-sib parents in the breeding herd as both cases involve both animals sharing half of their alleles (Ott, 1999). As a result, in a number of cases

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offspring were allocated to the true sire's father. This effect is expected to be much smaller in breeding herds with fewer sires (Sherman *et al*., 2004). Overall, the results from the parentage analysis were acceptable and no cases were left unresolved. Where ambiguous paternity occurred, the use of on-farm records provided confirmation of parentage allocations.

5.2 Microsatellite marker panel

The microsatellite marker panel used in the study was constructed in a previous study by Friedrich (2009), which was selected specifically for use in the parentage verification of South African Angora goats. A number of parameters that are routinely used for assessment of parentage marker panels were estimated and compared with the results published from previous similar studies as presented in Table 5.2.

The number of markers included in the study are within the recommended range (12 to 14 markers) for parentage studies, according to ISAG (www.isag.us). Previous parentage studies in goats made use of marker panels consisting of between nine and 18 microsatellites (Jimenez-Gamero *et al.,* 2006; Friedrich, 2009). The ISAG recommended panel for parentage studies consists of 16 markers, however, the study by Friedrich (2009), consisting of 14 microsatellites, created the final panel suitable for use in parentage testing specifically in the South African Angora goat population. The final panel utilized for the current study consisted of 12 microsatellite markers; two markers from the panel of Friedrich (2009), namely SRCRSP9 and SRCRSP24, failed to amplify during optimization and were therefore excluded from the study.

The marker panel for the current study performed better than previous studies in terms of polymorphic information content (PIC) as indicated by Table 5.2. PIC values give an indication of how informative a specific marker is (Liu & Cordes, 2004; Webster & Reichart, 2005). A high PIC may either be indicative of a considerably unrelated population or a highly heterozygous population (Altet *et al.,* 2001). Since PIC is calculated using the number of alleles per locus and the allele frequencies (Liu & Cordes, 2004), the high PIC in the current study is a result of the high level of polymorphism and distinguishing power of the microsatellite markers in the panel used. This is consistent with the relatively high H_E that was achieved. Altet *et al.* (2001) found that a marker panel in an inbred population with a lower PE but higher PIC performed similarly (regarding successful parentage assignment) to the same marker panel in an outbred population with higher PE.

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Table 5.2 Comparison of microsatellite marker panel from the present study to previous studies in terms of PE (probability of exclusion), PIC (polymorphic information content), HWE (deviation from Hardy-Weinberg Equilibrium), H_E (expected heterozygosity), H_O (observed heterozygosity) and mean number of alleles per locus

Study	Species	Number of individuals	Number of markers	CPE	PIC	HWE	H_E	Mean number of alleles per locus
Current	Goats	381	12	0.996	0.702	5	0.740	12.25
Visser *et al*, 2011	Goats	200	18	0.998	0.629	11	0.672	8.83
Van Eenennaam *et al.*, 2007	Cattle	649	23	0.999	0.629	0	NC	9.00
Bolormaa *et al.*, 2008	Goats	976	14	0.998	0.7	2	NC	8.15
Altet *et al.,* 2001	Dogs	360	10	0.956	0.401			
De Araújo *et al.*, 2010	Goats	292	11	0.988	0.542	NC	0.717	8.00
Jimenez-Gamero *et al.*, 2006	Goats	620	9	0.999	0.701	NC	0.733	9.78

PE: Probability of exclusion of sire when no parental genotypes are known
PIC: Mean Polymorphic Information Content for the entire panel
HWE: Number of markers in the panel that showed significant deviation from Hardy-Weinberg Equilibrium in the population
H_E: Estimated heterozygosity
NC: Not calculated in the study

The combined probability of exclusion for sire alone when no parental genotypes are known (CPE) was lower than studies of Van Eenennaam *et al.* (2007), Bolormaa *et al.* (2008), De Araújo *et al.* (2010), Jimenez-Gamero *et al.* (2006) and Visser *et al.* (2011) as indicated in Table 5.2. A lower CPE value is indicative of relatedness within the population. This is, however, to be expected as the population for the current study was selected from one breeder and is not a representation of the South African Angora goat population as a whole. The study by Visser & Van Marle-Köster (2009), in which a reference population of 1067 South African Angora goats was developed, determined that there was more variation than expected and very little inbreeding in the national herd.

Five markers showed significant deviation from Hardy-Weinberg Equilibrium (HWE). This is related to expected heterozygosity (H_E), in that deviation from HWE is a result of an excess of heterozygotes in the population (Altet *et al.,* 2001). Five of the 12 markers in the current study showed significant deviation from HWE, which was lower in proportion than Visser *et al.* (2011) (11 out of 18 markers showed

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deviation from HWE) and larger in proportion than van Eenennaam *et al.* (2007) and Bolormaa *et al.* (2008) where none of the 23 markers and two of the 14 markers showed deviation from HWE respectively. One reason for deviation from HWE is the presence of null alleles in a population (Karlsson & Mork, 2005).

Markers INRA 63, MCM 527 and BM 1258 showed a positive null allele frequency, while BM 1329 and INRABERN 192 showed negative null allele frequencies (Table 5.3). This suggests that the former three markers deviated significantly from HWE possibly as a result of null alleles in the population. Other reasons for deviation from HWE are an excess of heterozygotes in the population or association with a coding region that is under selection (Li & Leal, 2009). Markers BM 1329 and INRABERN 192 may have deviated from HWE as a result of excess heterozygotes or may be in linkage disequilibrium with a causative mutation. Of the five markers that showed deviation from HWE in the present study, only markers BM 1329, BM 1258 and INRABERN 192 showed significant deviation from HWE in the study of Visser *et al.* (2011).

Table 5.3 Deviation from HWE and null allele frequencies for microsatellite markers in the current study

Microsatellite marker	Null allele frequency	H_E
INRA 63	+0.046	0.772
MCM 527	+0.037	0.760
BM 1258	+0.016	0.789
BM 1329	-0.022	0.709
INRABERN 192	-0.069	0.629

H_E: Estimated heterozygosity

The microsatellite marker panel in the current study produced a higher average H_E in the population than previous studies. This is concurrent with deviation from HWE and a relatively higher PIC as shown in Table 5.2. One possible explanation may be that the breeder made use of sufficient unrelated breeding animals that significant variation was maintained in the herd, confirmed also by the high number of alleles per locus where the current study produced more alleles per locus than other studies compared in Table 5.2.

5.3 Estimated Breeding Values (EBVs) and sire ranking

The significance of parentage testing is realized in the effects on selection accuracy and genetic progress. This section will discuss the change in EBV estimation and EBV accuracy from the on-farm pedigree to

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the DNA-verified pedigree for seven traits of economic importance. Ultimately the greatest impact of the change in EBV estimation after the pedigree amendment lies in the highest and lowest producing sires as these animals would be most affected by selection and culling decisions. Therefore, the change in the sire ranking from the on-farm to the DNA-verified pedigree is discussed.

Performance testing in South African Angora goats, through the National Small Stock Improvement Scheme, has been available to producers since 1999 (Olivier & Snyman, 2011). Most farmers make use of a selection index for multitrait (fleece weight, fibre diameter and body weight) selection (Snyman, 2002; Snyman *et al.,* 2010; Visser & Van Marle-Köster, 2011) using phenotypic performance values (animal's own performance). There are currently two selection indices that are being used by Angora goat breeders (Snyman *et al.,* 1996). The first (S_1) will yield an increase in body weight, a decrease in fibre diameter and maintain a constant fleece weight:

$$S_1 = (3 \times BW) + (4 \times FW) - (23 \times FD)$$

The second (S_2) will yield an increase in body weight and maintain constant fibre diameter and fleece weight:

$$S_2 = (3 \times BW) + (15 \times FW) - (1 \times FD)$$

Although there is currently limited participation in the formal performance testing programme, selection index analyses are carried out at GADI at breeders' requests. Multiple trait selection by Best Linear Unbiased Prediction (BLUP) has been shown to yield better response to selection and genetic progress than mass selection on animals' own performance records (Belonsky & Kennedy, 1988; Dekkers, 1991; Quinton & Smith, 1995; Simm *et al.,* 2001). Another study by Sorensen (1987) on Danish pigs realised an increase of 29% with BLUP selection compared to a selection index. The reason for this advantage in BLUP selection is that pedigree information is taken into account (Belonsky & Kennedy, 1988).

EBV accuracies have been defined as the correlation between the estimated and true breeding value for a trait (Falconer & Mackay, 1996). The accuracy of an EBV is an indication of the confidence in the estimate and the potential genetic gain (response to selection) that may be made from relying on a particular estimate. The EBVs of the sires changed significantly in the DNA-verified pedigree, this was also reflected in the change in the ranking of the sires for each of the traits. Mixed response was observed

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in the accuracies of the EBVs for different traits. Vast improvements were seen in traits for sires E1, F1, G1, S1 and S11, owing probably to the allocation of more offspring during the parentage analysis.

It is important to select for fibre traits as they contribute to the quality of mohair produced (Snyman, 2002). This is especially important because mohair is a luxury fibre and the price is, therefore, determined by the quality of the fleece (Shelton, 1993; Snyman, 2002). The sires' EBVs in the current study for fleece weight ranged between -0.077 and 0.311. The best fleece weight of fine hair sires in the study by Snyman (2002) reported a mean EBV of -0.02 in 1999. Fibre diameter ranged between 4.86 and -1.12, which exceeded that reported by Snyman (2002), where average sire EBVs for fibre diameter was -1.07 in 1999. Birth weight EBVs ranged between -0.054 and 0.154, weaning weight EBVs ranged from -1.19 to 1.72, eight month weight from -0.23 to 1.85, yearling weight from -0.99 to 1.94 and 16 month weight ranged from -0.67 to 2.35.

Selection goals in Angora goat breeding have significantly evolved over the past few decades. In previous years breeders placed emphasis on fibre diameter, fleece weight and body weight, while fibre diameter had the greatest effect in determining the price of the clip (Shelton, 1993; Snyman. 2002). During the 1980s, the greatest selection emphasis was placed on fleece weight, while ignoring fibre diameter, which boosted the yield produced by South Africa during this time (Snyman, 2002). More recently breeders have made use of a selection index comprised of fleece weight, fibre diameter and body weight (Snyman *et al.,* 2010).

Fleece weight (FW) and fibre diameter (FD) are under intense selection by breeders in the Angora goat industry. Studies have shown that there is a positive, yet unfavourable, genetic correlation (r_g= 0.55) between FW and FD (Snyman *et al.,* 2011). The use of selection indices have made it possible to select for both FW and FD simultaneously, without disadvantaging either trait (Lush, 1947; Falconer, 1981).The impact of the DNA analysis was not as great on FW as on other traits included in the current study. Most sires that were ranked in the top and bottom five performing sires remained there in the DNA-verified pedigree (Table 4.9, CHAPTER 4: RESULTS). A reason for this could be that the magnitude of selection pressure that had been placed on FW in the past resulted in most sires in the herd having positive EBVs for the trait. The EBVs for FW in the on-farm pedigree appear to be underestimated when comparing them to the DNA-verified pedigree. This may be a result of an increased number of offspring in the dataset of the DNA-verified pedigree leading to an overall higher average in performance. This will reflect in an increase in the genetic trend for fleece weight as a result of amending DNA pedigrees in this herd.

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Similar effects can be seen for FD. Most top producing sires in the on-farm pedigree remained in the top producers of the DNA-verified pedigree and similarly in the lowest producers, with one exception. Sire S1 was ranked among the five lowest producers in the on-farm pedigree but in the top five of the DNA-verified pedigree. The EBV of sire S1 also improved drastically after the DNA analysis. Although the sire was only allocated one additional offspring during the DNA assignment, the EBV improved as a result as changes in the greater dataset (over 5000 records were used for the calculation of genetic parameters), not just from the single offspring.

Revised selection practices in South African Angora goats have taken body weight into account in selection indices owing to the correlation that has been found between body weight and fleece traits. Strong genetic correlations were found between body weight and both fleece weight and fibre diameter (r_g = 0.70 and 0.62 respectively) (Snyman *et al.,* 2011). The correlation between body weight and fibre diameter is positive and unfavourable. An increase in body weight would result in a correlated increase in fibre diameter, therefore incorporation of body weight and fleece traits into a selection index is the only way to maintain optimum performance in all three traits (Snyman *et al.,* 2010). Furthermore, larger body weights are associated with improved reproduction and survivability (Erasmus, 1987; Cronje, 1992; Shelton, 1993) (and an increased fleece weight) and, therefore contribute to the overall efficiency and welfare of the animals.

A greater effect of the change in pedigree was observed in the body weights. Definite trends were seen in terms of the highest and lowest sires that were correlated with the fleece trait ranked sires. Sires B1, S3, S1, S8, S4 and G1 showed a substantial decrease in their EBVs and were initially ranked in the top five performing sires for different body weights in the on-farm pedigree, but shifted in rank to the five lowest performing sires in the DNA-verified pedigree. Similarly, sires D1, S11, A1, G1, H1 and F1 that were ranked in the lowest five performers in the on-farm pedigree were ranked among the top five producing sires n the DNA-verified pedigree. This was most obvious in weaning weight, where sire A1, which was ranked lowest in the on-farm pedigree moved up to the top ranking position in the DNA-verified pedigree with an accuracy of above 90% (Table 4.9, CHAPTER 4: RESULTS). This particular sire is one of the oldest of the sires included in the present study (born in 2001) and sired many offspring in the time period 2000 – 2009 (period in which all offspring in the dataset used for EBV estimation in the current study were born), which accounts for the high accuracy achieved. Similarly, for birth weight, sire D1 was ranked lowest in the on-farm pedigree and was ranked second in the DNA-verified pedigree with an

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accuracy of 82%. This is a relatively young sire (born in 2006) but has shown above average performance in more than one trait.

A few sires appeared in the top five performing sires in the DNA-verified pedigree and hold potential in selection for a superior mohair clip. Sire D1 featured in the top five sires for FW, FD W0, W4 and W8. Sires A1 and C1 was ranked in the top five for W4, W8, W12 and W16. B1 and G1 were only ranked in the top five sires for FW and W4 respectively, however these sires have the highest number of offspring assigned to them and can be considered proven sires. These sires, as well as sire S11 for fibre diameter, would likely perform well when selecting for fleece weight, fibre diameter and body weight using BLUP EBVs.

By examining the results of highest and lowest producing sires in the on-farm pedigree and DNA-verified pedigree, conclusions can be made regarding the breeding strategy of the breeder and the potential genetic progress that has been forfeited. The breeder has selected eight sires (A1, B1, C1, D1, E1, F1, G1 and H1) for AI and single sire mating. After the EBV analysis and sires were ranked according to on-farm pedigrees group sires were found in some of the top five ranks. This is unexpected as breeders would normally select the top sires for AI and single-sire mating, not use them as group sires. This suggests that the breeder did not make use of EBVs in his selection strategies. Some of these sires are among the lowest performers in the DNA-verified pedigree for the different traits.

Selection indices using phenotypic performance values have been the preferred method of selection of South African Angora goat breeders even though a higher accuracy is known to be achieved with BLUP EBVs (Snyman *et al.,* 2010; Olivier & Snyman, 2011). However it is not certain as to whether the breeder made use of selection indices as there was limited participation in performance testing at the time of the study (personal communication: Dr M.A. Snyman, GADI, Middelburg, National Department of Agriculture, Eastern Cape).

The results of the current study show great potential for improvement of selection accuracy and potential response to selection, as well as financial returns. The study by Visscher *et al.* (2002) demonstrated that genetic gains of 2-3% could be achieved if pedigree errors of 10% were amended in UK dairy cattle. Israel & Weller (2000) determined that, after the correction of 10% errors in paternity records, genetic gains were 4.3% higher in Israli cattle herds. Gomez-Raya *et al.* (2008) ran a cost to benefit analysis for paternity testing in beef cattle in the United States and found that an additional return of between 70 – 140% may be made on money invested in DNA-based paternity testing with microsatellites. The reward

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of implementing DNA-based parentage verification has been demonstrated in previous studies in different species and is also possible for South African Angora goat breeders. It will also be of great benefit to the South African Angora goat industry if producers would participate in the national recording scheme and practice selection based on EBVs. The study by Snyman *et al.* (2010) estimated that one to two percent genetic progress may be made annually when selection is practiced using the selection indices designed specifically for the use in South African Angora goat breeding schemes, with a potential added gain of up to 20% by basing these indices on EBVs.

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CHAPTER 6: Conclusion

There are currently around 900 000 Angora goats in over800 farms in South Africa. Angora goat breeders often keep both stud and commercial herds on one farm, currently there are 44 registered stud breeders. South Africa is the largest mohair producer in the world, contributing more than half of the global production each year. In order to maintain the high standard of the mohair clip produced genetic progress must be made using every resource possible each year. Molecular research in South African Angora goats has advanced significantly in the last decade including studies by Visser (2011) and Friedrich (2009) and the generation of the bio-bank reserve for South African small stock at the Grootfontein Agricultural Development Institute (GADI) in the Eastern Cape (Snyman, 2011).

Unsound pedigrees have presented a challenge to selection accuracy in the past, which necessitated the creation of a DNA-marker panel for parentage verification. In the study by Friedrich (2009), a panel of 14 microsatellite markers was designed specifically for South African Angora goats, which was used in the current study. The final panel, consisting of 12 microsatellite markers in the current study, was validated in a population of 381 South African Angora goats. The panel was evaluated for P_E (for sire alone), PIC, H_E and H_O, deviation from HWE and null allele frequency. The P_E of the panel was slightly lower than expected as a result of a higher degree of relatedness in the population, a consequence arising from selecting the study population from one single breeder. PIC and heterozyosity in the population were acceptably high and the marker panel proved suitable for unambiguous parentage verification.

The pedigrees were arranged into half sib families for the 21 sires using CERVUS software.14.3% of the recorded pedigrees were found to be inaccurate or incomplete. A challenge in the study was relatedness of the sires in the herd. A few incidents occurred where kids were assigned to a relative of the true sire due to the sharing of common alleles between the related sire. On-farm records were used to confirm the parentage in each of these cases. This leads to either of two suggestions: either breeders should keep fewer related bucks as breeding males to reduce inbreeding (and allele sharing) in the herd or more markers could be included in the panel to increase the testing power for parentage of such herds. Furthermore, inbreeding can pose greater long term risks than inaccurate parentage alone. Inbreeding depression is associated with depressed longevity, lower production and higher incidence of disease in livestock herds and should be avoided at all costs. Pedigree integrity plays an important role in the prevention of inbreeding and DNA-based parentage verification may potentially be very helpful in the maintenance thereof.

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Currently there are no South African Angora goat breeders taking part in performance testing and therefore selection is being carried out based on the phenotypic performance of animals. It has been shown that selection based on EBVs is far more accurate than mass selection (Belonsky & Kennedy, 1988). The results from the current study show that the sires selected for AI (based on the on-farm pedigrees) were not all among the top five ranked sires based on EBVs for each trait, meaning that there were already inaccuracies in selection as a result of the decisions being based on mass selection rather than on EBVs. This effect is compounded by the pedigree inaccuracies identified by the DNA-based parentage testing. The reordering of the pedigrees by the microsatellite marker-based parentage verification had a profound effect on the estimation of EBVs and ranking of sires for the different traits considered in the study. In some instances, sires that were ranked as one of the lowest performers (based on EBVs for each trait) in the on-farm pedigrees were scored in the top five sires according to the DNA-based pedigree. In practice this may have resulted in the breeder culling one of his top sires based on an incorrect breeding value estimate as a result of incorrect pedigree recording. Conversely, the breeder may have been selecting a sire that was estimated as a top producer (based on recorded pedigrees) which, in reality, was one of the more inferior sires in the herd. A potential challenge in this method of selecting may be where sires produced very few offspring and the erroneous recording of one offspring may have a large effect on the EBV of the sire. In such cases it is important to consider the accuracy of the EBV. The accuracy is affected by the number of offspring belonging to each sire. EBVs with higher accuracies are more reliable owing to the number of offspring that were taken into account in the estimation of the EBV.

In conclusion, the implementation of DNA-based parentage verification using microsatellite markers is an opportunity for Angora goat farmers to make more accurate selection decisions and greater genetic gains. This is applicable to the use of mass selection in breeding programmes, however, incorporating the use of EBVs into breeding programmes holds greater potential for superior genetic gains and vastly improved selection accuracy.

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ADDENDUM A: Half-sib families according to DNA-based parentage assignments

Table 1A Parentage allocation of family A with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/06250	A1	A1	11	0	4.700	*
142/06257	A1	A1	11	0	5.090	*
142/06330	A1	A1	11	0	5.030	*
142/06331	A1	A1	11	0	4.440	*
142/06335	A1	A1	12	1	-2.030	*
142/06370	A1	A1	12	1	1.770	*
142/06379	A1	A1	10	0	3.190	*
142/06398	A1	A1	12	0	5.560	*
142/06412	A1	A1	12	0	7.030	*
142/06419	A1	A1	10	0	2.290	*
142/06431	A1	A1	12	0	2.020	*
142/06439	A1	A1	12	2	-3.600	*
142/06461	A1	A1	11	0	5.330	*
142/06470	A1	A1	12	1	-0.981	*
142/06471	A1	A1	12	0	3.610	*
142/06473	A1	A1	12	0	2.700	*
317/06122	A1	A1	12	1	1.820	*
317/06161	A1	A1	12	1	1.160	*
142/06373[a]	A1	**C1**	12	0	3.820	*
142/07245	A1	**C1**	10	1	0.145	*
142/07286	A1	**C1**	12	0	3.810	*
142/07298[a]	A1	**C1**	12	0	3.090	*
142/08295[a]	A1	**C1**	12	1	2.520	*
154/06628[a]	A1	**C1**	12	1	-2.360	*
215/08785	A1	**H1**	12	0	5.880	*
142/08363	A1	**None**	12	1	-0.780	*
317/08085	A1	**None**	12	0	6.020	*
142/06328[b]	H1	A1	11	1	1.820	*
317/06099[b]	H1	A1	11	1	-2.290	*
142/06366[b]	S11	A1	12	1	1.730	*
142/06437[bc]	S7	A1	11	1	-2.100	*
215/06870[bc]	S14	A1	11	1	-2.650	*
317/06140[bc]	S14	A1	12	0	1.240	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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Table 2A Parentage allocation of family B with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/06333	B1	B1	12	1	0.186	*
142/06360	B1	B1	12	1	2.030	*
142/06399	B1	B1	12	2	-0.830	*
142/06402	B1	B1	11	1	3.200	*
142/06415	B1	B1	12	0	5.590	*
142/06416	B1	B1	11	0	4.540	*
142/06434	B1	B1	10	0	3.160	*
142/06476	B1	B1	12	1	-1.690	*
142/07277	B1	B1	12	1	0.055	*
142/07315	B1	B1	12	0	10.900	*
142/08215	B1	B1	12	1	2.350	*
142/08220	B1	B1	12	1	2.280	*
142/08245	B1	B1	12	1	3.800	*
142/08246	B1	B1	12	1	3.430	*
142/08252	B1	B1	11	1	3.240	*
142/08269	B1	B1	11	1	1.830	*
142/08272	B1	B1	10	0	4.500	*
142/08277	B1	B1	10	0	5.260	*
142/08290	B1	B1	12	0	3.570	*
142/08291	B1	B1	12	0	7.520	*
142/08296	B1	B1	12	0	4.170	*
142/08301	B1	B1	12	1	1.430	*
142/08303	B1	B1	12	0	7.450	*
142/08331	B1	B1	12	0	8.560	*
142/08336	B1	B1	12	1	-0.734	*
142/08341	B1	B1	12	1	1.670	*
142/08343	B1	B1	11	0	5.770	*
142/08344	B1	B1	12	1	-0.297	*
142/08346	B1	B1	11	0	3.670	*
142/08347	B1	B1	11	1	1.630	*
142/08348	B1	B1	11	1	-1.580	*
142/08353	B1	B1	10	1	-0.353	*
142/08364	B1	B1	12	0	6.220	*
142/08374	B1	B1	12	1	1.340	*
154/06678	B1	B1	12	1	1.630	*
215/07626	B1	B1	12	1	0.702	*

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215/08717	B1	B1	9	0	2.840	*
215/08725	B1	B1	12	0	3.440	*
215/08727	B1	B1	12	1	1.150	*
215/08730	B1	B1	10	0	4.180	*
215/08731	B1	B1	12	0	2.360	*
215/08746	B1	B1	12	1	-0.835	*
215/08747	B1	B1	12	0	7.290	*
215/08756	B1	B1	12	1	1.490	*
215/08786	B1	B1	11	0	6.780	*
215/08787	B1	B1	11	0	6.500	*
317/08016	B1	B1	12	0	5.880	*
317/08017	B1	B1	12	0	4.510	*
317/08023	B1	B1	12	0	4.510	*
317/08027	B1	B1	11	0	5.560	*
317/08028	B1	B1	12	1	1.130	*
317/08029	B1	B1	12	1	1.240	*
317/08071	B1	B1	12	1	4.090	*
317/08072	B1	B1	11	1	2.380	*
142/08354	B1	H1	12	0	2.650	*
142/08398	B1	None	12	1	1.080	*
142/08404	B1	None	12	0	4.700	*
215/08802	B1	None	12	1	-0.977	*
215/08814	B1	None	12	0	6.020	*
142/08256[b]	S12	B1	12	4	-11.200	*
142/06332[bc]	S12	B1	12	1	-1.230	*
154/06627[bc]	S15	B1	12	2	-2.130	*
215/08748[b]	S1	B1	10	1	1.100	*
142/08234[b]	S5	B1	11	1	0.889	*
142/08251[b]	S5	B1	12	0	5.350	*
142/08314[b]	S5	B1	12	0	3.010	*
215/06855[b]	S5	B1	11	2	-3.720	*
215/08781[b]	S5	B1	11	0	3.780	*
317/07073[b]	S5	B1	10	0	3.540	*
317/08070[b]	S5	B1	12	0	5.150	*
317/08083[b]	S5	B1	12	1	1.980	*

[a] Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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Table 3A Parentage allocation of family C with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/06362	C1	C1	12	0	3.850	*
142/06408	C1	C1	12	0	3.890	*
142/07327	C1	C1	12	0	2.490	*
142/08288	C1	C1	12	0	5.640	*
142/08292	C1	C1	12	1	-1.390	*
142/08293	C1	C1	12	0	5.250	*
142/08306	C1	C1	11	1	3.140	*
154/06679	C1	C1	12	0	6.000	*
154/06682	C1	C1	12	0	7.260	*
215/06830	C1	C1	12	0	5.130	*
215/06837	C1	C1	12	0	7.180	*
215/06849	C1	C1	12	0	5.580	*
215/06850	C1	C1	12	0	5.850	*
215/07619	C1	C1	11	0	7.910	*
215/07827	C1	C1	12	1	0.154	*
215/07850	C1	C1	11	0	6.250	*
215/08734	C1	C1	9	0	3.060	*
215/08739	C1	C1	12	0	3.610	*
317/07016	C1	C1	12	2	-6.590	*
317/07029	C1	C1	12	0	5.770	*
317/07049	C1	C1	12	1	-0.503	*
317/07061	C1	C1	12	2	-4.600	*
317/07068	C1	C1	12	0	6.360	*
317/07069	C1	C1	12	0	5.690	*
317/08021	C1	C1	11	0	4.880	*
317/08022	C1	C1	12	0	2.180	*
142/08268	C1	**D1**	12	1	-0.130	*
215/08729	C1	**B1**	12	1	-0.814	*
317/08078	C1	**B1**	12	3	-8.580	*
142/06373[b]	A1	C1	12	0	3.820	*
142/07298[b]	A1	C1	12	0	3.090	*
142/08295[b]	A1	C1	12	1	2.520	*
154/06628[b]	A1	C1	12	1	-2.360	*
215/06856[bc]	S15	C1	12	0	5.840	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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Table 4A Parentage allocation of family F with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/06327	F1	F1	11	0	6.460	*
142/06340	F1	F1	12	1	2.880	*
142/06356	F1	F1	12	0	6.220	*
142/06380	F1	F1	12	0	2.080	*
142/06396	F1	F1	12	0	8.070	*
142/06401	F1	F1	12	0	5.880	*
142/06410	F1	F1	12	0	8.540	*
142/06423	F1	F1	12	0	6.940	*
142/07309	F1	F1	6	0	4.340	*
142/07321	F1	F1	12	0	9.550	*
154/06630	F1	F1	12	0	4.060	*
154/06680	F1	F1	12	0	3.950	*
215/06839	F1	F1	12	0	5.050	*
215/06848	F1	F1	12	0	11.000	*
215/06852	F1	F1	12	0	4.180	*
215/06859	F1	F1	12	0	4.590	*
215/06872	F1	F1	12	0	9.130	*
317/06095	F1	F1	12	0	7.290	*
317/06130	F1	F1	12	0	9.880	*
317/06149	F1	F1	12	0	4.310	*
317/07055	F1	F1	12	0	4.430	*
317/07057	F1	F1	12	0	4.740	*
317/07066	F1	F1	12	0	3.100	*
142/06364	F1	**A1**	12	0	11.700	*
142/08332	F1	**None**	11	1	3.270	*
215/08762	F1	**None**	12	0	2.310	*
215/08770	F1	**None**	12	0	4.050	*
317/08054	F1	**None**	12	0	12.200	*
317/08061	F1	**None**	12	1	4.120	*
142/06400[bc]	S7	F1	12	0	11.000	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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Table 5A Parentage allocation of family G with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/08223	G1	G1	12	0	4.330	*
142/06225	G1	G1	12	0	6.420	*
142/06240	G1	G1	12	0	6.180	*
142/06242	G1	G1	12	0	9.800	*
142/06247	G1	G1	12	0	8.010	*
142/06255	G1	G1	12	0	4.290	*
142/06275	G1	G1	12	0	9.460	*
142/06277	G1	G1	12	0	7.140	*
142/06294	G1	G1	12	0	4.320	*
142/06297	G1	G1	12	0	1.340	*
142/06324	G1	G1	12	0	8.720	*
142/08201	G1	G1	12	0	5.670	*
142/08202	G1	G1	12	0	5.000	*
142/08209	G1	G1	12	0	7.770	*
142/08210	G1	G1	12	0	5.330	*
142/08218	G1	G1	12	1	1.370	*
142/08221	G1	G1	12	0	8.030	*
142/08231	G1	G1	12	0	4.150	*
142/08232	G1	G1	12	0	7.150	*
142/08239	G1	G1	12	0	5.600	*
142/08248	G1	G1	12	0	6.910	*
142/08249	G1	G1	12	0	5.470	*
142/08254	G1	G1	12	0	7.270	*
142/08264	G1	G1	12	0	6.670	*
142/08266	G1	G1	12	0	7.340	*
142/08270	G1	G1	12	0	4.980	*
142/08274	G1	G1	12	0	3.060	*
142/08278	G1	G1	12	0	3.800	*
142/08286	G1	G1	12	0	7.840	*
142/08297	G1	G1	12	0	2.890	*
142/08302	G1	G1	12	0	6.550	*
154/06613	G1	G1	12	0	4.290	*
154/06621	G1	G1	11	0	4.050	*
154/06665	G1	G1	12	0	5.400	*
215/06803	G1	G1	12	0	8.300	*
215/06814	G1	G1	12	0	2.550	*
215/08702	G1	G1	12	0	7.690	*

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215/08710	G1	G1	12	0	0.738	*
215/08712	G1	G1	12	0	2.530	*
215/08716	G1	G1	12	0	6.420	*
215/08721	G1	G1	12	0	5.050	*
317/06009	G1	G1	12	0	4.740	*
317/06010	G1	G1	12	0	3.350	*
317/06018	G1	G1	12	0	3.850	*
317/06033	G1	G1	12	0	7.600	*
317/06034	G1	G1	11	0	2.730	*
317/06043	G1	G1	12	0	5.270	*
317/06044	G1	G1	12	0	3.960	*
317/06060	G1	G1	12	0	6.050	*
317/06069	G1	G1	6	0	3.170	*
317/06077	G1	G1	12	1	1.190	*
317/06097	G1	G1	11	0	6.110	*
317/08005	G1	G1	11	0	5.280	*
317/08012	G1	G1	11	0	3.940	*
317/08013	G1	G1	12	1	0.609	*
317/08019	G1	G1	12	0	1.960	*
317/08020	G1	G1	12	0	2.850	*
317/08036	G1	G1	12	0	6.680	*
317/08039	G1	G1	12	0	4.600	*
317/08043	G1	G1	12	0	7.050	*
142/08233	G1	**B1**	11	0	2.570	*
142/08367	G1	**None**	12	1	2.040	*
142/08368	G1	**None**	12	3	-5.720	*
142/08396	G1	**None**	12	1	-0.897	*
317/08001	G1	**None**	12	0	6.160	*
317/08002	G1	**None**	12	1	3.400	*
317/08090	G1	**None**	12	0	4.920	*
154/06605[b]	D1	G1	12	2	-6.380	*
215/08703[b]	S6	G1	12	3	-1.620	*
215/08719[b]	S6	G1	11	2	-0.625	*
317/08040[b]	S16	G1	12	2	1.380	*
317/06078[bc]	S14	G1	12	0	2.770	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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Table 6A Parentage allocation of family H with 12 microsatellite markers

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
142/08310	H1	H1	12	0	4.790	*
142/08312	H1	H1	12	0	6.410	*
142/08313	H1	H1	12	0	8.540	*
142/08342	H1	H1	12	0	6.440	*
142/08355	H1	H1	12	0	8.050	*
215/08745	H1	H1	12	0	9.720	*
215/08749	H1	H1	12	0	6.170	*
215/08755	H1	H1	12	0	8.430	*
215/08777	H1	H1	12	0	6.480	*
215/08784	H1	H1	12	0	3.830	*
317/07058	H1	H1	12	0	6.160	*
317/08045	H1	H1	12	0	7.040	*
317/08048	H1	H1	12	0	7.730	*
317/08050	H1	H1	12	0	5.880	*
317/08064	H1	H1	12	0	6.390	*
317/08065	H1	H1	12	0	5.310	*
317/08068	H1	H1	12	0	8.270	*
317/08069	H1	H1	12	0	6.820	*
317/08075	H1	H1	12	1	0.763	*
317/08079	H1	H1	12	0	7.270	*
317/08080	H1	H1	12	0	3.250	*
142/06328[a]	H1	**A1**	11	1	1.820	*
317/06099[a]	H1	**A1**	11	1	-2.290	*
142/07287[bc]	S8	H1	12	2	2.840	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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Table 7A Parentage allocation of group sire families (S1 to S16)

Offspring ID	Allocated sire ID	Recorded sire ID	Pair loci compared	Pair loci mismatched	LOD score	Confidence
215/08748[a]	S1	B1	10	1	1.100	*
142/08369[a]	S1	D1	12	2	0.979	*
142/08384	S1	None	12	1	-0.318	*
215/08753[a]	S2	D1	11	3	-4.630	*
142/08333	S2	None	12	3	-5.420	*
317/08084	S3	None	12	1	0.500	*
317/08046[a]	S4	D1	12	1	1.890	*
142/08385	S4	None	12	0	3.270	*
215/08810	S4	None	12	2	-5.080	*
317/08092	S4	None	12	0	4.890	*
142/08234[a]	S5	B1	11	1	0.889	*
142/08251[a]	S5	B1	12	0	5.350	*
142/08314[a]	S5	B1	12	0	3.010	*
215/06855[a]	S5	B1	11	2	-3.720	*
215/08781[a]	S5	B1	11	0	3.780	*
317/07073[a]	S5	B1	10	0	3.540	*
317/08070[a]	S5	B1	12	0	5.150	*
317/08083[a]	S5	B1	12	1	1.980	*
317/08100	S5	None	12	2	-4.430	*
317/08102	S5	None	12	0	2.170	*
215/06835	S6	A1	12	2	0.965	*
215/08703[a]	S6	G1	12	3	-1.620	*
215/08719[a]	S6	G1	11	2	-0.625	*
142/08366	S6	None	12	3	-5.240	*
142/06437[ac]	S7	A1	11	1	-2.100	*
142/08275	S7	C1	12	1	-0.812	*
142/06400[ac]	S7	F1	12	0	11.000	*
142/08273	S8	G1	12	3	-4.250	*
317/08011	S8	G1	11	0	8.990	*
142/07287[ac]	S8	H1	12	2	2.840	*
142/08338	S8	H1	12	2	1.030	*
142/06366[a]	S11	A1	12	1	1.730	*
154/06673	S11	E1	12	1	0.024	*
142/08323	S11	None	8	0	3.010	*
142/08372	S11	None	12	0	10.200	*
317/08107	S11	None	12	1	2.650	*
142/06332[ac]	S12	B1	12	1	-1.230	*

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142/08256[a]	S12	B1	12	4	-11.200	*
215/08778	S12	D1	12	3	-6.980	*
142/08324	S12	None	12	0	6.120	*
142/08330	S12	None	11	0	8.780	*
142/08373	S12	None	12	0	1.430	*
215/08765	S12	None	12	0	4.670	*
215/08771	S12	None	12	0	3.090	*
317/08093	S12	None	12	0	9.800	*
142/06301[ac]	S13	E1	12	2	0.632	*
142/08327	S13	None	12	3	-5.450	*
215/06870[ac]	S14	A1	11	1	-2.650	*
317/06140[ac]	S14	A1	12	0	1.240	*
215/07628	S14	B1	12	0	2.210	*
317/06078[ac]	S14	G1	12	0	2.770	*
215/08799	S14	None	12	1	1.840	*
154/06627[ac]	S15	B1	12	2	-2.130	*
215/06856[ac]	S15	C1	12	0	5.840	*
215/08705	S15	D1	11	0	6.540	*
142/06234[ac]	S15	E1	12	1	1.530	*
142/06260[ac]	S15	E1	10	0	5.280	*
215/06815[ac]	S15	E1	12	1	4.540	*
215/06824[ac]	S15	E1	12	1	2.880	*
142/08350	S15	None	12	2	-4.680	*
215/08798	S15	None	11	0	5.960	*
317/08040[a]	S16	G1	12	2	1.380	*
317/08101	S16	None	12	2	0.852	*

[a]Kids to be removed from family and assigned to another sire after cross-referencing with on-farm data
[b]Kids reassigned to family after cross-referencing with on-farm data
[c]Kids that were allocated to sires that were less than two years older than them by Cervus and were reallocated to the on-farm recorded sire

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ADDENDUM B: Facilities for DNA-based parentage testing in South Africa

Inqaba Biotech™ (Pty) Ltd

Address: 459 Leyds St, Pretoria 0002

Phone: +27 12 343 5829

www.inqababiotec.co.za

Onderstepoort Veterinary Genetics Laboratory

Address: University of Pretoria
Faculty of Veterinary Science
Old Faculty Building
Room 2 - 8
Onderstepoort
0110

Phone: +27 12 529 8240

www.up.ac.za

Unistel Medical Labratories (Pty) Ltd

Address: Faculty of Health Sciences
Clinical Building, Suite 13
Francie can Zijl Drive
Tygerberg
7505

Phone: +27 21 938 9213/4

www.unistelmedical.co.za

Agricultural Research Council (ARC) Irene Animal Production Institute: Animal Genetics

Address: Olifantsfontein road
Irene
25º53'59.6"S ; 28º12'51.6"E

Phone: +27 12 672 9111

www.arc.agric.za

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