# GENETIC CHARACTERIZATION OF <br> SOUTHERN AFRICAN SHEEP <br> BREEDS <br> USING DNA MARKERS 

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by

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## ABSTRACT

Merino sheep are an important resource for South Afric an farmers, providing meat and wool and thus an important income source. Indigenous and locally developed breeds are an important asset for many reasons, but particularly because, over time, they have developed unique combinations of adaptive traits to respond to the pressures of the local environment. To be able to distinguish between breeds for conservation and utilization purposes, the genetic variability, population structure and phylogenetic relationships were determined. Seven different Merino genotypes were sampled. These included the Dormer, SA Merino, SA Mutton Merino, Landsheep, Letelle, Dohne and Afrino. The indigenous and locally developed breeds comprised of the Damara, Pedi, Blinkhaar Ronderib Afrikaner, Blackhead Persian, Blackhead Speckled Persian, Redhead Persian, Redhead Speckled Persian, Zulu, Namaqua Afrikaner, Karakul, Swazi, Van Rooy and Dorper.

The Merino, indigenous and locally developed breeds were assessed for genetic diversity using 24 microsatellites. Different statistical analyses were performed to determine the genetic variation, genetic relationships and genetic differentiation of the breeds.

The SA Merino showed a high number of very distinct alleles. This study confirmed a higher variability of the SA Merino when compared with the other breeds. The genetic distance between the SA Merino and SA Mutton Merino, both fine wool breeds, was high indicating that these two breeds are relatively distant from each other. The Afrino known to have 25\% SA Merino, $25 \%$ Ronderib Afrikaner and $50 \%$ SA Mutton Merino, indicated a closer relationship with the SA Mutton Merino. This result confirmed the development of the breed. From the phylogenetic analysis between the seven Merino genotypes, when compared to the other estimates obtained in the study, it was evident that the Merino genotypes in South Africa have more within breed variation than between breed variation.

The genetic distance estimates observed for the indigenous fat-tailed breeds were relatively high indicating that even between these breeds genetic differences exist. As expected, a smaller genetic distance between the Persian varieties was observed. Genetic distances between the developed breeds supported their ancestral
development. The results of the indigenous and locally developed breeds present the first study of the genetic characterization of these breeds using microsatellite markers in South Africa.

Southern Africa is hosting a very large sheep (Merino, indigenous and locally devebped) genetic resource. Adapted to the agricultural production systems of the continent, it represents a unique resource that has great potential for further development of its productivity.

## PREFACE

Two positive outcomes were achieved with this study:
i) The achievement of genetic characterization of farm animal genetic resources in the Southern African Development Community (SADC) region. The knowledge gained from this study enabled the application of the techniques for determination of the genetic variation and genetic structure of populations and breeds important for the conservation and utilization of southern African indigenous sheep breeds.
ii) The application of the molecular tools used in this study contributed to the establishment of a routine parentage verification service to the sheep industry in South Africa. Another valuable national contribution is the techniques developed and standardized molecular testing methods through this study that contribute to the solving of stock theft cases for the South African Police Services.

## Introduction



## CHAPTER ONE

## 1. GENERAL INTRODUCTION

Domestication of livestock by man introduced a major cultural revolution. Hominids and early man were hunters and gatherers for millions of years. The climatic fluctuations, which followed the end of the glacial period some 14000 years ago, may have been instrumental in forcing man to domesticate animals. Records of the domestication of sheep dates lack as early as 7000 BC in the Near East (Plug \& Badenhorst, 2001).

Sheep are thought to have evolved from the goat-antelope, Rupicaprini represented by the Capriconis of Southeast Asia. This has been supported by paleontology and behavioral evidence (Geist, 1971). During the late Pleistocene period, goats and sheep formed an interbreeding population (Payne, 1968). Blood antigens, blood proteins and chromosome structure showed large differences between sheep and goats (Lay et al., 1971). The fact that present-day interbreeding between sheep and goats is not very successful makes such a link unlikely (Dain, 1970; Curtain, 1971; Lindley et al., 1971). Wild sheep have survived in large numbers despite the presence of man. The home of the wild sheep is the mountain ranges of Central Asia, from where sheep spread westward into Europe and eastwards into North America during the Pleistocene period (Ryder, 1983).

The taxonomic status of the genus Ovis is open to dispute. There are six species of wild sheep in existence, which could have given origin to our domestic breeds (Ryder, 1983, 1984). The most important of these are the Argali (Ovis ammon), the Urial (Ovis orientalis), the Mouflon (Ovis musimon) and the Bighorn (Ovis canadensis). All domestic breeds of sheep are thought to have descended from the Mouflon (O. musimon), although the Urial (O. orientalis) may have contributed to European breeds.

Domesticated sheep have $2 \mathrm{n}=54$ chromosomes, the same chromosome number as the European mouflon, the Asiatic mouflon, the Bighorn and the Dall sheep. The snow sheep of eastern Siberia has 52 pairs of chromosomes, the Argali, 56 and the Urial, 58. (Ryder, 1984; FAO, 2000). It is unclear whether these chromosomal
differences represent the cause of speciation and domestication (Short, 1976). It is believed that the Urial was domesticated first, since Urial remains have been found around the area where domestication appeared to have begun (Ryder, 1984). The Urial is thought to have arrived in Europe first and later the Mouflon. The two species are thought to have mixed (Ryder, 1983). It is also believed that the Urial gave rise to "wool" sheep and that "hair" sheep originated from the Mouflon (Zeuner, 1963). Others believe that only a single wild species contributed to the gene pool of present day domestic sheep. This theory has been supported by chromosome counts and blood protein analysis (Schmidtt \& Ulbright, 1968; Ryder, 1984). In recent studies by Hiendleder et al. (1998; 2002), the origin of domesticated sheep was investigated using mitochondrial DNA (mtDNA). Sixteen mtDNA haplotypes among 243 domesticated sheep of European, Asian and Central Asian origin were identified. None of these haplotypes were present in Urial or Argali, thus excluding these two species as ancestors of present-day domesticated sheep. However, some of these haplotypes were found in wild populations of Mouflon, strongly indicating that this species has contributed to the genetic pool of domesticated sheep.

There are a number of different theories regarding the origins of domestic sheep. Sheep (Ovis aries) were among the earliest livestock species to be domesticated. As ruminants, they provided humankind with a means of digesting via fermentation, a substantial proportion of the fibrous material produced by grasslands, which singlestomach or monogastric species are less able to digest. Sheep ( $O$. aries) evolved in Eurasia in the early Pleistocene period about 2.5 million years ago (Ryder, 1983). The first sheep that appeared in the Villafranchian period were as large as oxen. By the end of this period, approximately three million years ago, the first true sheep replaced these 'oxen.'

African sheep breeds migrated with various nations from Asia, Arabia and the Middle East into North Africa. Epstein (1971) stated that a number of nomadic black and colored tribes inhabited North Africa many years ago. A tsetsefly breakout stretching along the equator across the whole of Africa restricted these nations from migrating southward. According to archaeological records, sheep migrated to the Cape as recently as 2000 years ago (Plug \& Badenhorst, 2001).

Southern Africa is commonly referred to as 'a world in one country.' The rich culture of the various population groups and nations, the wealth of native flora and fauna and animal genetic resources in particular, endow this subcontinent with a variety and attractiveness unequalled throughout the world. It also has the unique benefit of indigenous species and breeds of livestock which sustained ancient pastoralists in their migration down the African continent many centuries ago.

The conservation of domestic animal diversity is essential to meet future needs in Africa and Southern Africa. In order to cope with an unpredictable future, genetic reserves capable of readily responding to directional forces imposed by a broad spectrum of environments must be maintained. Maintaining genetic diversity is an insurance package against future adverse conditions. Due to diversity among environments, nutritional standards and challenges from infectious agents, a variety of breeds and populations are required. These act as storehouses of genetic variation which forms the basis for selection and may be dawn upon in times of biological stress such as famine, drought or disease epidemics. The wide range of breeds and species are each specifically adapted to a different set of conditions.

In addition, with increasing global human population pressures, the quantity of food and other products must increase. Not only should diversity be maintained for practical purposes, but also for cultural reasons. A community's domestic animals can enhance the environment as a living system, thus also enhancing the human inhabitant's quality of life.

The Khoi-Khoi people possessed three species of animals, namely, Zebu type cattle, fat-tailed sheep and dogs (Plug \& Badenhorst, 2001). Their migration occurred during the 14th and 15 thcenturies AD. When they reached Southern Africa, they migrated southwards along the West Coast of Angola, Namibia and Namaqualand, until they reached the southern tip of Africa. South Africa has its own unique sheep genetic resources that include wool breeds originally imported as well as indigenous and locally developed breeds.

Indigenous and locally developed sheep breeds are an important asset for many reasons, but particularly because, over time, they have developed unique combinations of adaptive traits to best respond to pressures of the local environment (see breed descriptions in Appendix 1). In this study the indigenous breeds include
the Damara, Karakul, Pedi, Blackhead Persian, Blackhead Speckled Persian, Redhead Persian, Redhead Speckled Persian, Zulu, Swazi, B linkhaar Ronderib Afrikaner and Namaqua Afrikaner. The locally developed breeds comprised of the Dorper and Van Rooy. They have adapted and settled in a variety of biomes.

These adaptive traits include (Hammond, 2000):

* tolerance/resistance to various diseases,
* tolerance to fluctuations in availability and quality of feed resources and water supply,
* tolerance to extreme water temperatures, humidity and other climatic factors,
* adaptation to low capacity management conditions and
* ability to survive, produce and reproduce for long periods of time.

The Merino breeds are important contributors to the wool and meat industry in South Africa (see breed descriptions in Appendix 1). By now, many of these breeds have been selected to adapt to the South Afric an climatic conditions. Rege et al. (1996) classified the Merino breeds from South Africa in two main groups viz. fine wool and developed wool breeds. The fine wool breeds include the SA Merino, originally imported from Spain and the SA Mutton Merino. The developed breeds are grouped into coarse wool (Dormer) and fine wool breeds (Afrino, Dohne Merino and Letelle). In this study, the fine wool and developed breeds within the Merino are referred to as 'Merino genotypes'. The Afrino, Dohne, Dormer and SA Mutton Merino are locally developed Merino breeds. A study to support existing phenotypic characterization and the conservation of developed breeds using molecular markers has now become important.

The need for conservation comes from the potential rate of decrease of genetic variation. The loss of genetic variation within and between breeds is detrimental not only from the perspectives of culture and conservation but also utility since lost genes may be of future economic interest. Within breeds, high rates of loss of genetic variation leads to reduced chances of breed survival due to decreased fitness through inbreeding depression. These breeds become subject to faster changes in gene frequencies, greater rate of loss of genes and genetic constitutions (haplotypes). These are all due to small, effective population sizes, or, equivalently, high rates of inbreeding (Meuwissen, 1991).

Once animal genetic diversity has been lost, it cannot be replaced. Advances in biotechnology offer possibilities of improving, utilizing and conserving present domestic animal diversity. The economic implications of maintaining existing farm animal genetic resources in their natural environment are negligible as compared to the costs involved in biotechnology development (FAO, 2000).
Animals, as compared to plants, are more complicated and more expensive to manipulate. Animals have hundreds of thousands of genes which interact in a complex way (Weller, 2001). It is this unique combination of genes, their interaction with each other and the environment that determines an animal's ability to produce or adapt itself to a particular environment, and it is our insurance against an unpredictable future.

The Food and Agricultural Organization (FAO) of the United Nations has proposed a global programme for the management of genetic resources using molecular methodology for breed characterization (Bjornstad \& Roed, 2001). This strategy places a strong emphasis on the use of molecular markers to assist the conservation and assessment of endangered breeds and to determine the genetic status of these breeds.

The study of the structure and function of genes at the molecular level in a breeding population can help determine the similarity of the genetic material carried by populations and the genetic variation they possess. Several techniques have been developed to estimate the genetic variation or polymorphisms in populations and hence the genetic relationship amongst populations. These methods include biochemical polymorphisms, immunological methods and molecular methods (DNA hybridization, RFLP, RAPDS, mtDNA, microsatellites and SNP). The advantages and disadvantages of some of these methods will be discussed.

### 1.1 Blood typing and protein polymorphisms

Blood and protein polymorphisms were used during the 1960's but revealed a limited number of loci and alleles at a locus (Nei, 1987; Tanabe et al., 1991). This method is rapid, affordable and reliable, but requires fresh blood samples. Detection of prdein polymorphisms involves the electrophoretic separation of proteins based on the differences of their molecular weight followed by histochemical recognition of
differences in banding patterns for particular proteins between individuals (Baker et al., 1966).

The first study published using protein markers to determine the genetic variation of sheep breeds from South Africa was conducted by Sargent (2000). From the six enzyme systems Albumin (Alb), Diaphorase (Dia), Carbonic Anhydrase (CA), Haemoglobin (HB), Transferrin (TF) and protein (X) analyzed, the D-allele was the most common allele at the TF system for most of the breeds, except for the SA Mutton Merino and Karakul breeds, the H -allele was found only in the Dormer breed, although at a low frequency, and the Gallele was evident only in the Afrino, Van Rooy, Blackhead and Speckled Persian breeds. All the breeds except SA Mutton Merino and Van Rooy, were monomorphic at the ALB system. An A-allele was present in the SA Landrace, Afrino and Namaqua Afrikaner breeds. All the breeds studied had the B-allele as the most common allele at the ALB, CA and X loci. Allele frequencies of SA Merino at the TF-locus were compared to allele frequencies of Merino breeds in other countries. The SA Merino differed from the other breeds at the same locus. The theoretical basis of the study of polymorphic proteins is that breeds can be defined as populations that differ from each other in the relative distribution and frequencies of genes (Hasselholt, 1969). The confirmation of some of these results using DNA technology has now become important.

### 1.2 DNA Hybridization

DNA hybridization was developed in the 1960's and was the first technique used to study the organization of eukaryotic genomes and was applied in molecular evolution and systematics studies (Sibley \& Ahlquist, 1990). After denaturation, DNA from two genomes is combined and allowed to re-anneal on cooling. The extent of nucleotide differences between the two different strands can then be approximated upon reheating and measuring the temperature at which the double strands dissociate, providing an index of relatedness and conversely of genetic distances. This technique has been used to estimate the genetic distance between species of higher primates and carnivores ( O ' Brien et al., 1985) as well as birds (Sibley \& Ahlquist, 1990). The technique was found unsuitable for obtaining the aims of this study.

### 1.3 Restriction fragment length polymorphisms (RFLP's)

In RFLP, genomic DNA is isolated, cut using restriction enzymes, sizefractionated on gels and transferred to a filter by blotting (Southern, 1975) and probed with clones from the genomic region of interest (Aquadro et al, 1992). The advantage of RFLP's is that it can be used to screen a large number of individuals without requiring complicated molecular techniques (Aquadro et al., 1992). In the case of this study, RFLP's were found to be less applicable and of the older DNA technologies.

### 1.3 Random amplified polymorphic DNA (RAPD)

RAPD (Williams et al., 1990) is a polymerase chain reaction (PCR) based technique that has been used for the study of populations. It uses one short oligonucleotide ( $\pm 10-12 \mathrm{bp}$ long) to amplify random segments of DNA. The polymorphisms generated by this technique indicate dominant-recessive characters (presence or absence of a band). This technique has been used successfully in the study of plants (Kantanen et al., 1995) but was found to be not highly reproducible in animals.

### 1.5 Mitochondrial DNA (mtDNA)

In animal cells, DNA is also found outside the nucleus in small oblate bodies known as the mitochondria. The reason for the increased use of mtDNA in population studies is that it is transmitted only through the maternal line in most species (Avise et al., 1987; Gyllensten et al., 1985; 1991), evolves more rapidly than nuclear DNA (Brown, 1985; Stoneking et al., 1991), is considerably smaller than nuclear DNA with a size of approximately 15-20 kilobases (kb) in length, comprises approximately 37 genes (Wallace, 1986), is present in multiple copies in each eukaryotic cell, there is general conservation of gene order and composition (Wilson et al., 1985) and is easily isolated and purified (White et al., 1998). The fact that mtDNA shows haplotype diversity within species makes it a useful tool in establishing phylogenetic relationships at or below the species level (Avise et al, 1987). mtDNA has been used in studies of the origins of sheep (Hiendleder et al., 1998; 2002). There are however a few drawbacks of using mtDNA for population
studies. The lack of recombination makes the mitochondrial genome a single heritable unit; potentially producing gene diversity estimates that have larger standard errors than those determined using nuclear loci (Dowling et al., 1990). An occasional bi-parental inheritance has been reported (Hoeh et al., 1991) that could also complicate mtDNA analysis.

### 1.6 Microsatellite markers

Molecular genetics is revealing new facets of genetic variation, both on the standing variation and on the new variation generated by mutations. Microsatellites are a new class of marker that has become the preferred technique for population studies. Microsatellites are short tandem repeats (STR's) of genomic sequences. The repeated unit can be a mono-, di-, tri- or tetranucleotide with di- repeats being most common. They generally occur in non-coding regions of the genome. DNA microsatellite sequences are valuable genetic markers due to their dense distribution in the genome, they are highly variable, co-dominant inherited and relatively easy to detect. As hypervariability is highly significant for detecting differences in a population and between individuals, microsatellite typing can reveal degrees of polymorphism that is easy to interpret. Microsatellites offer several advantages. They are relatively easy to isolate in different species, different loci can be used according to the level of variation, which ranges from very low to extremely high (Beaumont \& Bruford, 1999), they can be easily amplified by PCR and thus used on a wide range of sample material such as blood, hair, meat, saliva and skin, and their genetic systems are easily automated enabling the analysis of a large number of samples (Heyen et al., 1997; Luikart et al., 1999).

However, microsatellites do have several disadvantages. These include reports that for certain groups of organisms they are difficult to isolate (Beaumont \& Bruford, 1999), the technical challenges of microsatellite analysis for some types of samples such as saliva, hair or faecal material (Gerloff et al., 1995; Taberlet et al., 1996; Gagneux et al., 1997) and that data generated in different laboratories using different methods have proved difficult to amalgamate (Beaumont \& Bruford, 1999).

After standardization through international societies within different laboratories, the use of microsatellites for genetic characterization of livestock including cattle
(MacHugh et al., 1997; Hanotte et al., 2000; 2002; Hanslik et al., 2000; Megan et al., 2000), goat (Chenyambuga, 2002), camels (Nolte, 2003), horses (Botha, 2001) and sheep (Buchanan et al., 1994; Crawford \& Littlejohn, 1998; Parsons et al., 1996) is now accepted world wide. The popularity of microsatellites remains undiminished, as most researchers are of the opinion that the advantages such as the resolving power, outweighs their disadvantages. Microsatellite markers were chosen for this study as they are available and are recommended by the Food and Agricultural Organization (FAO) for animal genetic resources studies and as the Irene laboratory participates in the standardization comparison test of the International Society for Animal Genetics (ISAG).

### 1.7 Single nucleotide polymorphisms (SNP's)

DNA microarrays or "chips" have been used in studies ranging from gene expression to identification of single nucleotide polymorphisms (SNP's) or differences in DNA sequences amongst genotypes (Wang et al, 1998). The microarray technology allows the simultaneous analysis of thousands of parameters within a single experiment, thus generating large amounts of genomic data within a single experiment (Templin et al., 2002). The potential use of DNA chips and SNP's in the characterization of livestock has already been identified (Altshuler et al., 2000). At present, except for a few model organisms in which extensive genetic studies and genome sequencing projects that are in place, the unavailability of SNP markers in most species remains an obstacle to their systematic employment in population genetic studies (Zhang \& Hewitt, 2003). This technique is unaffordable at this stage, but should be kept in mind for future sheep genetic resources studies.

## 2. AIMS OF THE STUDY

To be able to distinguish between breeds for conservation and utilization purposes, the determination of the genetic variability, population structure and phylogenetic relationships using DNA microsatellite markers led to the formulation of the different aims for this study.

1. The first aim was to establish microsatellite marker sets for the genetic characterization of sheep. This included the selection, optimization and application of microsatellite markers for sheep in the ARC-Irene laboratory.
2. The second aim was to apply findings from the first aim to determine the genetic variability, genetic relationship and genetic differentiation within and between the Merino genotypes from South Africa. With the development of new breeds this is important since the influence of applied selection can be monitored within the breeds.
3. With the recent emphasis on conservation and the importance of biological diversity, also seen in the context of international animal genetic resources programmes the third aim was to compare the genetic variation, genetic relationships and genetic differentiation within and between the indigenous and locally developed breeds from South Africa.

## Material and Methods



## CHAPTER TWO

## MATERIAL AND METHODS

Blood and hair were sampled from a total of 640 sheep comprising 20 breeds from different regions in Southern Africa (Table 1). Animals from different localities were sampled to ensure that each population was representative of the breed (Figure 1). The particular location was to some extent also indicative of the migration routes of sheep into Southern Africa. The aim was to sample 10 unrelated males and 30 unrelated females. This however was not always possible for all the breeds sampled.

Table 1: Sheep breeds sampled, numbers, type, and location

| Breed | n | Type | Location |
| :---: | :---: | :---: | :---: |
| Damara | 34 | Fat-tailed | Northern Cape |
| Pedi | 40 | Fat-tailed | Gauteng, Northern Province |
| Blinkhaar Ronderib Afrikaner | 35 | Fat tailed | Namibia, Eastern Cape |
| Blackhead Persian | 19 | Smooth-haired fat rumped | Northern Cape, Namibia |
| Blackhead Speckled Persian | 33 | Smooth-haired fat rumped | Northern Cape |
| Redhead Persian | 27 | Smooth-haired fat rumped | Northern Cape, Namibia |
| Redhead Speckled Persian | 26 | Smooth-haired fat rumped | Northern Cape |
| Nguni (Zulu) | 35 | Fat-tailed | Kwazulu Natal |
| Namaqua Afrikaner | 34 | Fattailed | Eastern Cape |
| Karakul | 30 | Fat-tailed | Upington |
| Swazi | 27 | Fat-tailed | Swaziland |
| Van Rooy | 32 | Fat-tailed | Northern Cape |
| Dorper | 23 | Mutton | Eastern Cape |
| Dormer | 35 | Mutton | Western Cape |
| SA Merino | 30 | Wool | Eastern Cape |
| SA Mutton Merino | 35 | Mutton and Wool | Eastern Cape |
| SA Landsheep Letelle | $\begin{aligned} & 40 \\ & 34 \end{aligned}$ | Mutton and Wool Wool | Free State <br> Eastern Cape |
| Dohne | 40 | Wool | Eastern Cape |
| Afrino | 19 | Wool | Eastern Cape |



1 Dormer
2 SA Merino 3 SA Mutton 4 SA Landsheep
5 Lettele 6 Dohne
7 Afrino

Figure 1a: Map indicating localities of Merino genotypes sampled


1 Damara
2 Karakul
3 Pedi
4 Blinkhaar
Ronderib
Afrikaner
5 Van Rooy
6 Blackheard
Persian
7 Blackhead Speckled Persian
8 Redhead Persian
9 Redhead Speckled Persian 19 Zulu 11 Swazi 12 Namaqua Afrikaner 13 Dorper

Figure 1b: Map indicating localities of indigenous and locally developed breeds

For the purpose of this study, only blood samples were used. Whole blood was collected from each animal in 7 ml Vacutainer tubes containing the anticoagulant, ethylenediaminetetra-acetic acid (EDTA). The blood samples were kept cold and caution was taken to prevent exposing them to extreme temperatures. The hair samples collected from most of the breeds were stored in the Irene Bio-Store and used as a back-up.

DNA was extracted from the whole blood with the Wizard $®$ Genomic DNA Purification Kit (Miller et al., 1998) (Figure 2).


Figure 2: The Wizar』® Genomic DNA Purification Kit to extract DNA from blood

The following procedure was followed:

- For $300 \mu \mathrm{l}$ sample volume, $900 \mu \mathrm{l}$ cell lysis solution was added. Samples were incubated for 10 minutes at room temperature and centrifuged at 13000-16000 x g for 20 seconds.
- Supernatant was discarded leaving a visible white pellet.
- $300 \mu 1$ nuclei lysis solution was added
- $100 \mu \mathrm{l}$ protein precipitation solution was added and centrifuged for three minutes at 13000-16000x g.
- The clear supernatant was transferred to a clean 1.5 ml microcentrifuge tube containing $300 \mu \mathrm{l}$ isopropanol.
- The tube was gently inverted and white thread-like strands were visible.
- This tube was centrifuged for one minute at $13000-16000 \mathrm{x}$ g.
- The supernatant was decanted and $300 \mu 170 \%$ ethanol was added to the DNA pellet and centrifuged for another minute at $13000-16000 \mathrm{xg}$.
- The ethanol was carefully aspirated and the pellet was allowed to air dry for 10-15 minutes.
- $100 \mu \mathrm{l}$ DNA rehydration solution was added and the DNA was rehydrated by leaving the solution overnight at room temperature.
- Extracted DNA was stored at $-20^{\circ} \mathrm{C}$ until analysis in the polymerase chain reaction (PCR)
A total of 31 microsatellite markers were screened with a panel of five individuals. The microsatellites included: INRA006, TGLA126, ETH10, RM004, ILSTS002, BM1824, TGLA122, OARCP49, TGLA48, MAF214, INRA231, CSSM31, OARFCB11, BM1818, MAF65, BM1258, SRCRSP5, BM1329, CSRD247, ILSTS087, INRA23, TGLA53, SPS115, INRA63, CSSM36, MGTG4B, OARFCB20, TGLA57, MCM527, ETH225, and HSC. Following screening, only 24 microsatellites were found suitable for analysis in this study (Table 2).

Table 2: Characteristics of selected microsatellites

|  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| BM1824 | 1 (Bovine) | 155-195 | F: GAGCAAGGTGTTTTTCCAATC <br> R: САТТСТССАAСТGСТTССТTG | Bishop \& Kappes. (1994) |
| TGLA57 | 1 (Ovine) | 80-120 | F: GCTTTTTAATCCTCAGCTTGCTG <br> R: GCTTCCAAAACTTTACAATATGTAT | Steffen \& Eggen. $(1993)$ |
| INRA231 | 2 (Caprine) | 144-180 | F: AACATTTCAGCTGATGGTGGC <br> R: TTCTGTTTTGAGTGGTAAGCT | Kemp et al. (1993) |
| OARFCB20 | 2 (Ovine) | 60-120 | F: AAATGTGTTTAAGATTCCATACAGTG <br> R: GGAAAACCCCCATATATACCTATAC | Buchanan \& Crawford. (1992) |
| INRA23 | 3 (Bovine) | 190-240 | F: GAGTAGAGCTACAAGATAAACTTC <br> R: TAACTACAGGGTGTTAGATGAACTCA | Vaiman $\quad \& \quad$ Mercier. (1994) |
| MGTG4B | 4 (Ovine) | 120-145 | F: GAGCAGCTTCTTTCTTCTCATCTT R: GCTCTTGGAAGCTTATTGTATAAAG | Steffen \& Eggen. $(1993)$ |
| ETH10 | 5 (Bovine) | 180-220 | F: GTTCAGGACTGGCCCTGCTAACA <br> R: ССТССАGСССАСТТТСТСТТСТС | Solinas Toldo \& Fries. (1993) |
| MCM527 | 5 (Ovine) | 155-195 | F: GTCCATTGCCTCAAATCAATTC <br> R: AAACCACTTGACTACTCCCCAA | Hulme et al. (1994) |


| ILSTS087 | 6 (Ovine) | 130-175 | F: AGCAGACATGATGACTCAGC <br> R: CTGCCTCTTTTCTTGAGAGC | Kemp et al. (1993) |
| :---: | :---: | :---: | :---: | :---: |
| TGLA48 | 7 (Ovine) | 145-170 | F: AAATGTTTTATCTTGACTACTAAGC <br> R: ACATGACTCTGCCATAGAGCAT | Kempet al. (1993) |
| ETH225 | 9 (Bovine) | 120-160 | F: GATCACCTTGCCACTATTTCCT <br> R: ACATGACAGCCAGCTGCTACT | Steffen \& Eggen. $(1993)$ |
| ILSTS002 | 14 (Ovine) | 120-150 | F: TCTATACACATGTGCTGTGC <br> R: CTTAGGGGTGAAGTGACACG | Kemp et al. (1993) |
| MAF65 | 15 (Ovine) | 110-140 | F: AAAGGCCAGAGTATGCAATTAGGAG <br> R: CCACTCCTCCT GAGAATATAACATG | Buchanan et al. (1992) |
| RM004 | 15 (Ovine) | 100-160 | F: CAGCAAAATATCAGCAAACCT <br> R: CCACCTGGGAAGGCCTTTA | Kossarek \& Grosse. (1993) |
| SPS115 | 15 (Bovine) | 220-280 | F: AAAGTGACACAACAGCTTCTCCAG <br> R: AACGAGTGTCCTAGTTTGGC TGTG | Kemp et al. (1993) |
| MAF214 | 16 (Ovine) | 175-205 | F: GGGTGATCTTAGGGAGGTTTTGGAGG <br> R: AATGCAGGAGATCTAGGCAGGGACG | Buchanan \& Crawford. (1992) |
| TGLA53 | 16 (Bovine) | 130-175 | F: CAGCAGACAGCTGCAAGAGTTAGC <br> R: CTTTCAGAAATAGTTTGCATTCATGCAG | Crawford et al. (1995) |
| INRA63 | 18 (Bovine) | 165-225 | F: ATTTGCACAAGCTAAATCTAACC <br> R: AAACCACAGAAATGCTTGGAAG | Vaiman \& Mercier. (1994) |
| SR-CRSP5 | 18 (Caprine) | 142-164 | F: GGACTCTACCAACTGAGCTACAAG <br> R: TGAAATGAAGCTAAAGCAATCC | Arevalo et al. (1994) |
| TGLA126 | 20 (Bovine) | 105-135 | F: CT AATTTAGAATGAGAGAGGCTTCT <br> R: TTGGTCTCTATTCTCTGAATATTCC | Kempet al. (1993) |
| BM1818 | 23 (Caprine) | 230-275 | F: AGCTGGGAATATAACCAAAGG <br> R: AGTGCTTTCAAGGTCCATGC | Bishop $\&$ $(1994)$ |
| CSSM31 | 23 (Caprine) | 120-140 | F: CCAAGTTTAGTACTTGTAAGTAGA <br> R: GACTCTCAGCACTTTATCTGTGT | Moore \& Byrne. (1994) |
| CSSM36 <br> CSRD247 | (Ovine) unassigned (Caprine) unassigned | $150-210$ $220-260$ | F: GGATAACTCAACCACACGTCTCTG <br> R: AAGAAGTACTGGTTGCCAATCGTG <br> F: GGACTTGCCAGAACTCTGCAAT <br> R: CACTGTGGTTTGTATTAGTCAGG | Kemp et al. (1993) <br> Kemp et al. (1993) |

The microsatellite loci were selected on the basis of the degree of polymorphism and genome coverage as suggested by Barker et al. (2001). The selected microsatellite markers complied with the recommendations of the FAO and the International Society for Animal Genetics (ISAG). As stipulated by the Working Group of the FAO, microsatellite loci that can be used on several species such as cattle, sheep and goats are preferable. This aspect was also taken into account with the selection of the markers for this study. A total of eight cattle and five goat cross
species markers were therefore included in this study. The elimination of seven of the markers was due to the breakdown, inhibition of other markers in a plex and increased volumes required for optimum performance. The final 24 markers were successfully divided into four multiplexes based on product size and dye label (Table $3)$.

Table 3: Microsatellite markers and plexes

| Multiplex | Microsatellite loci |
| :--- | :--- |
| PLEX 1 | SPS115, TGLA53, INRA63, CSSM36, MGTG4B, OARFCB20, TGL A57, MCM527, ETH225 |
| PLEX 2 | CSRD247, ILSTS087, RM004 |
| PLEX 3 | INRA231, SRCRSP5, BM1818, MAF214, MAF65, TGLA126 |
| PLEX 4 | ETH10, BM1824, CSSM31, INRA23, TGLA48, ILSTS002 |

PCR reactions were prepared in four multiplexes. This minimized the chances of non-specific amplification. The PCR amplification was performed in a total volume of $7.5 \mu \mathrm{l}, 10 \mu \mathrm{l}, 12 \mu \mathrm{l}$ and $12 \mu \mathrm{l}$ respectively for each plex. This contained 2.5 mM dNTP's, 20 mM Tris- HCl with 15 mM MgCl , Supertherm Gold, deionized water, primer $(50 \mathrm{pmol} / \mu \mathrm{l})$ ) and $0.5 \mu \mathrm{l}$ extracted DNA. The amount of deionized water primer varied between each primer. The amplification was performed using a Perkin Elmer Gene Amp PCR System 9700 thermocycler (Figure 3). The amplification consisted of 15 min . at $95^{\circ} \mathrm{C}, 35$ cycles of 45 sec . at $94^{\circ} \mathrm{C}, 35$ cycles of 45 sec . at $59^{\circ} \mathrm{C}$ annealing temperature, 1 minute at $72^{\circ} \mathrm{C}$ and a final extension step at $72^{\circ} \mathrm{C}$ for 60 minutes. An ovine control DNA sample was included in each PCR. The ovine control DNA serves to indicate a problem with the PCR, or with the sample DNA. It also allows for the monitoring of the sizing accuracy since its sizing and labeling is known.


Figure 3: A thermal cycler

Genotyping was carried out on an automated ABI 377 DNA sequencer (Perkin Elmer, Foster City, USA; Figure 7), with fragments separated using a 5\% Long Ranger/6M gel (Figure 4). The internal size standard Genescan ${ }^{\mathrm{TM}}-350$ ROX $^{\mathrm{TM}}$ (Figure 6) size standard was used. For a 36 cm gel, a 50 ml gel solution was used including 5ml of Long Ranger solution, 10ml of 10X Tris Boric Ethylenediaminetetra acetic acid (TBE) buffer and 18g urea. Deionized water, ( 35 ml ), was added and the solution gently mixed until all the urea crystals were dissolved. A $0.2 \mu$ cellulose nitrate filter was used to filter the solution. The filtrate was de -gassed for 5 minutes. Ammonium persulphate ( $250 \mu \mathrm{l}$ of a $10 \%$ solution) and $25 \mu \mathrm{l}$ tetramethylethylenediamine (TEMED) was added to the solution and the gel poured. The gelwas allowed to polymerize for at least 2 hours (Figure 5).

Before loading, the PCR product was diluted with $110 \mu 1$ deionized water. From this diluted mixture, $1 \mu \mathrm{l}$ was added to $3.0 \mu \mathrm{l}$ of loading mix (Figure 6) which consisted of $0.35 \mu \mathrm{l}$ Genescan ${ }^{\mathrm{TM}}$ - 350 ROX size standard, $1.3 \mu \mathrm{l}$ loading buffer ( 50 mM EDTA, $50 \mathrm{mg} / \mathrm{ml}$ blue dextran) and $1.0 \mu \mathrm{l}$ formamide. The samples were then preheated at $96^{\circ} \mathrm{C}$ for 2 minutes and immediately cooled on ice. A volume of $1.5 \mu \mathrm{l}$ of the sample mix was then loaded onto the gel.

The ge 1 conditions were as follows:

| Electrophoresis Voltage | $: 1 \mathrm{KV}$ |
| :--- | :--- |
| Electrophoresis Current | $: 11.8 \mathrm{~mA}$ |
| Electrophoresis Power | $: 11 \mathrm{~W}$ |
| Gel Temperature | $: 51^{\circ} \mathrm{C}$ |
| Laser Power | $: 40 \mathrm{~mW}$ |
| Running Time | $: 2$ hours |

The gel was allowed to run for 2 hours before analysis. After approximately twenty minutes, bands were visible on the gel (Figure 8). The data were collected by Genescan ${ }^{\mathrm{TM}}$ analysis software (version 3.1, Applied Biosystems) and the allele sizes were determined with the Genotyper ${ }^{\mathrm{TM}}$ software (version 2.0, Applied Biosystems).


Figure 4: Chemical composition of gels


Figure 5: Gel Plates


Figure 6: Loading Mix


Figure 7: The ABI Sequencer


Figure 8: Image of a Sheep Gel

The statistical analyses included the application of several computer programmes. Microsatellite toolkit was used for the mean number of alleles and gene frequencies (http://oscar.gen.tcd.ie/sdepark/ms-toolkit/index.html). The POPGENE version 1.31 (Yeh \& Yong, 1999) programme was used to determine Hardy-Weinberg equilibrium and whether there were any significant deviations from Hardy-Weinberg equilibrium, the genetic differentiation with the calculation of pair-wise Fst and gene flow (Nm) values and the genetic diversity within and between the breeds. Linkage disequilibrium was determined with the GENEPOP version 3.3 (Raymond \& Rousset, 1995) programme. The mean number of alleles detected in each population and the expected heterozygosities are good indicators to determine the genetic polymorphism within populations.

The distance method of Goldstein et al. (1995) and Slatkin (1995) were used with the programme POPULATION version 1.2.26 (http://www.cnrs-gif.fr/pge) to obtain the input files for the constructing of trees A neighbour-joining tree and a UPGMA tree were constructed for each group. Bootstrap resampling included 1000 replicates
to test the robustness of the topology of the trees. The trees were then viewed by using the TREEVIEW version 1.6.6(http://taxonomy.zoology.gla.ac.uk/rod/rod.html) programme.

The GENECLASS programme (Cornuet et al., 1999) was used to classify individuals into specific populations or breeds. Using this programme individuals, which are genetically similar, are assigned to the same cluster.

## Results and Discussion



## CHAPTER THREE

## RESULTS AND DISCUSSION

The results have been divided into two parts. The first part deals with the genetic variability and genetic relationships within and between the Merino genotypes while the second part deals with the genetic variability and genetic relationships between and within the indigenous and locally developed sheep breeds of Southern Africa.

The following aspects were addressed in this study:
i) Firstly, the calculation of the mean number of alleles and heterozygosity values to determine the extent and distribution of the genetic diversity within the Merino as well as within the indigenous and locally developed breeds.
ii) secondly, the genetic relationship between the breeds was determined by using the genetic distances.
iii) thirdly, phylogenetic trees were constructed to determine the genetic differentiation through calculating Fst and Rst values and
iv) fourthly, individuals were assigned to specific groups using assignment tests.

## Merino genotypes

A breed with constant gene and genotype frequencies is said to be in Hardy Weinberg equilibrium (HWE) (Falconer \& Mackay, 1996). The first step was to verify whether the genotypes studied were in Hardy-Weinberg equilibrium (HWE). Single locus tests for HWE were conducted for the seven genotypes with 24 markers. Table 4 indicates that there were some genotypes with several loci that deviated significantly from HWE (level of significance $\mathrm{P}<0.05$ ). Out of the locus-population comparisons performed, $12.5 \%$ deviated from HWE. This was much higher than the $5 \%$ expected by cha nce alone. It is difficult to pinpoint the exact cause of these deviations. The three markers INRA63, TGLA57 and ILSTS087 were found to
deviate from HWE. Although these three markers indicated a deficiency and excess of heterozygotes, this does not explain the deviation from HWE. It is known that the natural processes of mutation, migration, non-random mating, genetic drift and both artificial and natural selection are factors that are known to cause deviations from HWE. Of the 24 loci there were none that deviated from HWE for all the genotypes. Seven out of 8 genotypes had HWE deviations at loci SPS115 and CSRD247. Three out of 8 genotypes deviated from HWE at 12 loci.

All loci indicated either deficiency or excess of heterozygotes. Inra63, ETH225 and MAF65 indicated a higher level of deficiency of heterozygotes amongst all the markers. CSRD247, CSSM31 and TGLA53 indicated the highest level of excess heterozygotes. ETH225 was also found to be significantly out of HWE across Merino populations in a study by Diez-Tascon et al. (2000). These authors assumed that the most likely explanation was the identification of null alleles.

Table 4: Results of Hardy-Weinberg equilibrium for Merino genotypes and loci

|  | $\begin{aligned} & n \\ & \cdots \\ & n \\ & n \\ & n \end{aligned}$ |  |  |  |  | す  <br> $\sum_{i}$ $\sum_{n}^{\infty}$ <br> $\sum_{m}^{\infty}$  |  |  | $\begin{aligned} & \bar{r} \\ & \underset{y}{c} \\ & \underset{Z}{2} \end{aligned}$ |  | $\begin{aligned} & \bar{m} \\ & \sum_{0}^{2} \\ & 0 \end{aligned}$ | $\begin{aligned} & \pi \\ & \underset{\sim}{c} \\ & \underset{Z}{z} \end{aligned}$ | $\begin{aligned} & \stackrel{\circ}{4} \\ & \stackrel{3}{\partial} \\ & \hline \end{aligned}$ | $\sum_{\infty}^{\infty}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | * | * |  |  |  | * | * |  |  |  |  | * |  |  |  |
| 2 |  |  |  |  |  | * | * | * |  |  | * | * | * |  | * |
| 3 | * |  | * |  |  |  |  | * | * |  |  |  | * | * | - |
| 4 | * | * * | * * |  |  |  | - | * * | * | * | * |  | , |  |  |
| 5 |  | * | * | * | * | * | * | * | * | * | * |  |  | * | * |
| 6 |  |  |  |  |  | * | * * | * * |  |  | * | * | * | - | * |
| 7 |  |  |  |  |  |  |  | * |  | * | * |  |  |  | * |

* indicates loci in Hardy-Weinberg equilibrium

1-Dormer; 2-SA Merino; 3-SA Mutton; 4-SA Landsheep; 5-Letelle; 6-Dohne; 7-Afrino

Linkage disequilibrium, also known as gametic phase equilibrium or allelic association, is a measure of the association of alleles on gametes or chromosomes. A breed or genotype is considered to be in linkage disequilibrium at a set of loci if the alleles are not randomly assorted in the next generation but are inherited together as a unit. Linkage disequilibrium can be generated by genetic drift, mutation, admixture and selection.

The linkage disequilibrium tests within each of the seven genotypes were performed for the 266 pairwise combinations of the 24 loci. The Genepop program
determines linkage disequilibrium of each allele for each individual at a single locus in a pairwise comparison. On average, $3.60 \%$ of the locus pairs were in linkage disequilibrium within the eight genotypes assuming a $1 \%$ type 1 error rate $(\mathrm{P}<0.01)$. Assuming a $5 \%$ type 1 error rate ( $\mathrm{P}<0.05$ ) $6.12 \%$ of the loci were in linkage disequilibrium (Table 5). In a study by Mugai (2002) on average $4.11 \%$ of the locus pairs were in linkage disequilibrium at the $5 \%$ level using 9 loci.

The mean number of alleles (MNA) detected in each breed or genotype and the expected heterozygosities are good indicators of the genetic polymorphism within the breed under study. The MNA is the average number of alleles observed in a breed, while the expected heterozygosities are the proportion of heterozygotes observed in a breed (Nei, 1987).

The genetic variability within the Merino genotypes was determined calculating the mean number of alleles and heterozygosities (Table 6). The most polymorphic markers were CSRD247 and ILSTS087 with 16 alleles respectively. The least polymorphic loci were MAF214 and MGTG4B with 5 alleles respectively. The mean percentage of polymorphic loci (PPL) of $95 \%$ is relatively high and indicates the usefulness of these markers in population studies.

Table 6: Sample size, mean number of alleles (MNA), percentage of polymorphic loci (PPL), expected heterozygosity $\left(\mathrm{H}_{\mathrm{E}}\right)$ and observed heterozygosity $\left(\mathrm{H}_{\mathrm{O}}\right)$

| Breed | $\mathbf{n}$ | $\mathbf{M N A}$ | $\mathbf{P P L}$ | $\mathbf{H}_{\mathbf{E}} \pm$ s.e. | $\mathbf{H}_{\mathbf{O}} \pm$ s.e. |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 35 | 5.17 | 100 | $0.616 \pm 0.026$ | $0.561 \pm 0.018$ |
| 2 | 30 | 5.96 | 100 | $0.662 \pm 0.030$ | $0.555 \pm 0.019$ |
| 3 | 35 | 5.79 | 100 | $0.678 \pm 0.024$ | $0.562 \pm 0.018$ |
| 4 | 40 | 6.42 | 100 | $0.698 \pm 0.025$ | $0.531 \pm 0.017$ |
| 5 | 34 | 5.00 | 88 | $0.605 \pm 0.036$ | $0.389 \pm 0.019$ |
| 6 | 41 | 6.04 | 100 | $0.649 \pm 0.037$ | $0.522 \pm 0.017$ |
| 7 | 19 | 4.11 | 79 | $0.571 \pm 0.045$ | $0.431 \pm 0.029$ |
| Mean |  | 5.34 | 95 | $0.634 \pm 0.034$ | $0.451 \pm 0.021$ |

1-Dormer; 2-SA Merino; 3-SA Mutton; 4-SA Landsheep; 5-Letelle; 6-Dohne; 7-Afrino
A total of 267 alleles were observed in the seven genotypes. Allele frequencies across all loci ranged from 0.122 to 0.912 (Appendix 2). For the loci BM1824, ETH225 and OARFCB20 DiezTascon et al. (2000) reported 5, 7 and 13 alleles respectively. In this study 7, 9 and 11 alleles were observed for the same loci. Twelve, 12 and 9 alleles for TGLA53, MAF65 and ILSTS002 respectively were observed which are higher than that reported by Arranz et al. (2001) indicating 9, 5 and 7 alleles for the same loci. Farid et al. (1999) also reported on TGLA53 and observed 6 alleles. The same number of alleles for MAF65 (12) were also found by Buchanan et al. (1994).

Diez-Tascon et al., (2000) observed in their study allele differences between the Merino populations regarding selection criteria. The Spanish, New Zealand and

Portuguese Black populations have been selected for wool characteristics, whereas the French, German and Portuguese White populations have been selected on the basis of early maturing lamb for meat consumption. The fact that microsatellites are presumed to be neutral, the differences in allelic frequencies found between these groups of populations could be explained on the basis of selection criteria. It is thus likely that some markers can be linked to genes for meat and wool traits (DiezTascon et al., 2000).

Private alleles defined in this study as alleles unique to a single breed were observed in the following breeds: Dormer (4), SA Merino (26), SA Mutton Merino (8), SA Landsheep (7), Letelle (4), Dohne (5) and Afrino (2). Some of the rare alleles observed in the SA Merino were in high frequencies $(0.621)$. The gene frequencies observed for the private alleles range d from 0.012 to 0.621 (Appendix 2). For the SA Merino many distinct alleles were observed that can be indicative of possible breed markers. SRCRSP-5, MCM527 and TGLA57 are markers that can distinguish between the SA Merino and Dohne. ETH10, CSSM31, TGLA48 and CSRD247 can be regarded as breed specific loci between SA Merino and SA Mutton Merino. Alternative alleles within OARFCB20 and BM1824 are specific for SA Mutton Merino. It was interesting to observe private alleles at two loci (BM1818 and INRA231) in the composite Dormer breed that can distinguish it from SA Merino. Although low frequencies for these alleles were observed it can possibly distinguish between German and Spanish types. The SA Merino is developed in a completely different direction than from the Spanish Merino.

Hughes et al. (unknown) used a total of 27 microsatellite markers of which 5 corresponded to this study. They observed private alleles at the following loci: INRA63 (2), ETH225 (1), CSSM31 (1), ILSTS087 (1) and ILSTS002 (2). In this study, 4, 5, 2, 4 and 2 private alleles were observed for the same loci respectively. Most of the private alleles reported by these authors were observed in the Scottish Blackface while the SA Merino showed the highest number of private alleles in this study.

The mean number of alleles ranged from 4.11 in the Afrino to 6.42 in the SA Landsheep. In general the mean number of alleles was high as would be expected as the markers were specially selected for being highly polymorphic (Table 6). The

Letelle was the least diverse genotype with a MNA of 4.11. This low value can be explained as the MNA is highly dependent on the sample size. The standard deviation of the MNA varied between 1.91 and 2.39. The most diverse genotypes were the SA Landsheep and the Dohne with a MNA of 6.42 and 6.04 respectively. A study by Arranz et al. (1998) using 19 microsatellite markers indicated a MNA of 9.90 for the Spanish Merino. A MNA of 5.96 was observed for the SA Merino. This value is much lower than expected as it is well known that the SA Merino is of Spanish origin.

The $\mathrm{H}_{\mathrm{E}}$ ranged from 57\% in Afrino to $70 \%$ in Landsheep. $\mathrm{H}_{\mathrm{O}}$ ranged from $39 \%$ in Letelle to $56 \%$ in SA Mutton Merino (Table 6). The average $\mathrm{H}_{\mathrm{E}}$ ( $63 \%$ ) was higher in all genotypes as compared to the $\mathrm{H}_{\mathrm{O}}(45 \%)$. The overall Ho values were lower than values obtained from other studies. Arranz et al. (1998) observed a heterozygosity value of 0.771 for the Merino in a study of the genetic relationships amongst Spanish sheep. Heterozygosity values of 0.712 and 0.762 were observed for German and Spanish Merino respectively (Diez-Tascon et al., 2000). The difference in variability between the South African and European Merino genotypes can possibly be explained by the choice of microsatelite markers. Sample error that included possible related animals within a genotype should not be discarded. When compared to other Spanish breeds the Merino was the most variable (Arranz et al., 2001). This study also confirmed higher variability of the SA Merino when compared with the other genotypes.

The genetic relationships between breeds can be measured by determining the genetic distance between the breeds. This difference between the two breeds can provide a good estimate of how diverge nt they are genetically (Avise, 1994). The genetic relationships between the genotypes were determined with Nei's (1978) standard genetic distance ( $\mathrm{D}_{\mathrm{S}}$ ). In this study only Ds was determined as Mugai (2002) indicated no differences between Nei's $\mathrm{D}_{\mathrm{A}}$ index of genetic distance and $\mathrm{D}_{\mathrm{S}}$. Other authors also made use of Ds estimates (Arranz et al.,1998; Buchanan et al.,1994; Diez-Tascon et al., 2000). In all cases the $\mathrm{D}_{\mathrm{A}}$ values confirmed results obtained with $\mathrm{D}_{\mathrm{S}}$ although bootstrap values of the latter were lower. The matrix for distance estimates is shown in Table 7. The genetic distance estimates ranged from 0.172 (SA Mutton Merino and Dohne) to 0.855 (SA Merino and Letelle). The close similarity of
the SA Mutton and Dohne Merino can be the result of gene flow from a common source.

Table 7: Genetic identity (above diagonal) and genetic distances (below diagonal) between Merino genotypes

| Genotypes | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | **** | 0.498 | 0.796 | 0.700 | 0.471 | 0.745 | 0.527 |
| 2 | 0.698 | **** | 0.553 | 0.603 | 0.425 | 0.637 | 0.446 |
| 3 | 0.228 | 0.593 | **** | 0.790 | 0.551 | 0.842 | 0.695 |
| 4 | 0.357 | 0.506 | 0.235 | **** | 0.546 | 0.810 | 0.649 |
| 5 | 0.753 | 0.855 | 0.597 | 0.605 | **** | 0.583 | 0.482 |
| 6 | 0.294 | 0.451 | 0.172 | 0.211 | 0.539 | **** | 0.677 |
| 7 | 0.637 | 0.808 | 0.365 | 0.433 | 0.729 | 0.390 | **** |

1-Dormer; 2SA Merino; 3-SA Mutton; 4SA Landsheep; 5-Letelle; 6-Dohne; 7-Afrino
The distance between the SA Merino and SA Mutton Merino (0.593), indicate that these two genotypes are relatively distant from each other. The genetic distance between the Dormer (coarse-wool developed breed with German Merino influence) and the SA Merino (fine wool breed with Spanish influence) was 0.698 . This estimate indicates that these breeds are genetically different. As there are no fixed standards for genetic distances, results from different studies are interpreted according to the distance coefficient used and in accordance with he other statistical values used in the studies. Within the developed fine wool breeds (Afrino and Letelle) a genetic distance estimate of 0.729 was observed. The smallest genetic distance, within the fine wool breeds, was observed between the Afrino and Dohne (0.390). The developed breeds that include the Dormer and Letelle indicated a genetic distance estimate of 0.753 . The developed coarse wool breed (Dormer) was genetically closer to the SA Mutton Merino (0.228).

Although estimates for the distances were not determined within geographic regions, some interesting results were evident (Table 7). The Afrino known to have 25\% SA Merino, 25\% Ronderib Afrikaner and 50\% SA Mutton Merino, indicated a closer relationship with the SA Mutton Merino (0365) than with the SA Merino (0.808). These results confirmed the development of the breed. This breed adapted well in the north-west Karoo. The Dohne selected from SA Merino and SA Mutton Merino indicated a closer relationship with the SA Mutton Merino (0.172). According to the geographical distribution of the Dohne Merino in the Eastern Cape this result indicates a high SA Mutton Merino influence. The Dormer showed a close
relationship with the SA Mutton Merino (0.228). This result supports the history of the development of the breed (Swart, 1967).

Diez-Tascon et al., (2000) observed a genetic distance estimate of 0.209 between the Spanish and German Merino. A small genetic distance was observed between the French Mutton and German Mutton Mer ino (0.139) (Arranz et al., 1998). Buchanan et al., (1994) indicated in his study that the Merino breeds were unrelated from the British sheep breeds. They observed the smallest genetic distance between the Australian and New Zealand Merino, while Arranz et al., (1998) reported a small genetic distance between the Spanish and Portuguese Black Merino (0.086). In this study the genetic distance estimate of 0.593 between the SA Merino and SA Mutton Merino indicates a possible differentiation between wool and meat characteristics. These results support findings reported in the literature.

The genetic distances obtained are supported by the Fst estimate across all loci (0.218). The level of breed differentiation was low indicating that only $22 \%$ of the total genetic variation was explained by breed differences. The least genetically differentiated population pairs were SA Mutton and Dohne with a Fst value of 0.044 (Table 8). This result confirms the development of the Dohne from the SA Mutton Merino. The highest genetic differentiation was observed between the SA Merino and the Afrino, and the Dormer and Afrino with Fst values of 0.238 and 0.237 respectively. The Afrino breed was found to be the most genetically different. It is an accepted fact that the SA Merino displays more differences within the breed than in the breeds developed from it (Personal communication, Prof G. Erasmus, June 2004). Since genetic differentiation of breeds is higher when Fst values are closer to one, the values obtained in this study indicate low genetic differentiation among the breeds. These results are thus supported by the other estimates observed in the study. Since Fst yielded similar estimates as Rst (Goodman, 1997), results of the latter are not presented.

Table 8: Genetic differentiation (Fst-below diagonal) and Gene Flow (Nm-above diagonal) in the Merino genotypes

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{1}$ | $* * *$ | 1.659 | 4.021 | 2.900 | 1.021 | 3.133 | $\mathbf{0 . 8 0 2}$ |
| $\mathbf{2}$ | 0.131 | $* * *$ | 2.103 | 2.473 | 0.995 | 2.437 | 0.806 |
| $\mathbf{3}$ | 0.059 | 0.106 | $* * *$ | 4.675 | 1.165 | $\mathbf{5 . 5 0 3}$ | 1.047 |
| $\mathbf{4}$ | 0.079 | 0.092 | 0.051 | $* * *$ | 1.197 | 1.797 | 1.018 |
| $\mathbf{5}$ | 0.197 | 0.201 | 0.177 | 0.173 | $* * *$ | 1.171 | 0.811 |
| $\mathbf{6}$ | 0.074 | 0.093 | 0.044 | 0.050 | 0.176 | $* * *$ | 1.201 |
| $\mathbf{7}$ | $\mathbf{0 . 2 3 8}$ | $\mathbf{0 . 2 3 7}$ | 0.193 | 0.197 | 0.236 | 0.172 | $* * *$ |

1-Dormer; 2-SA Merino; 3-SA Mutton; 4-SA Landsheep; 5-Letelle; 6-Dohne; 7-Afrino
The gene flow ( Nm ) was the lowest between the Afrino and the Dormer (0.802) while the highest value of 5.503 was obtained between the SA Mutton and Dohne Merino. Values above one indicate progressively more gene flow between populations whereas values below one suggest interrupted gene flow and possible differentiation. The 5.5 migrants per generation observed in this study correspond to the genetic distance values and other measures obtained. The genetic structure of a breed at any time is the result of a balance between genetic drift and gene flow. Farid et al. (1999) observed complex patterns of gene flow in most British sheep breeds and suggested that these breeds may have been kept longer in isolation. When that result is compared to that obtained in this study it is evident that the South African Merino breeds were not subjected to isolation for long periods.

The phylogenetic relationship between the seven Merino genotypes was determined from the Ds genetic distance estimates. Both the neighbour-joining (Figure 9) and the UPGMA (Figure 10) methods were used to obtain the trees. The unrooted trees obtained showed some differences from each other. The low bootstrap values obtained in the neighbour-joining tree suggested that the robustness of the tree was not high, and that the topology obtained was less consistent. The numbers at the juncture of two branches are the percent of 1000 bootstrap trees with the same structure. The analyses indicate three clusters. The one cluster contained the SA Landsheep and Afrino. The second cluster indicated the Dormer and Letelle clustering together while the third cluster had the SA Mutton, SA Merino and Dohne Merino. The topology of the UPGMA tree indicated small differences when compared to that of the neighbour-joining tree. The one cluster consisted of the Letelle, SA Mutton and

Dormer, another with the Afrino while the third cluster consisted of the Dohne, SA Landsheep and SA Merino. Both trees indicate the SA Merino as a separate trunk. The SA Merino being of Spanish origin clusters separate from the German influence. It was also observed by Diez-Tascon (2000) that the French Mutton and German Mutton Merino clustered together. The closest relation was between the Spanish and Portuguese Black population. Neither the unrooted UPGMA nor the neighbourjoining trees distinguished between meat and wool types. Taking the topology of the trees in consideration and comparing this with the other estimates observed in this study it indicates that the Merino genotypes in South Africa have more within breed variation than between breed variation.


Figure 9: Unrooted Neighbour-joining tree representing the genetic relationships between seven Merino genotypes.


Figure 10. Unrooted UPGMA tree representing the genetic relationships between seven Merino genotypes.

Individual specific analysis was determined with the GENECLASS programme (Cornuet et al., 1999). The Bayesian method yielded the best results with $97 \%$ of the individuals being assigned to the correct source population (227 individuals of 234). With the assignment of individuals to a population a P value of less than 0.05 was used. This threshold is regarded as accurate for this study. The results indicated 2 individuals in the Dormer breed assigned with SA Mutton Merino, 1 individual in the SA Mutton Merino assigned with SA Landsheep and 1 individual from the Afrino assigned with the Dohne (Appendix 3). For the abovementioned breed individuals were assigned to single breeds. In the SA Landsheep and Dohne individuals were assigned to two different breeds. One individual was assigned to Dohne Merino in SA Landsheep, while one individual was assigned to Afrino and one to Landsheep in Dohne. The genotypes with a $100 \%$ correct assignment were the SA Merino and Letelle. Four individuals within breeds were mis-assigned to the Dohne Merino. In general, all the genotypes had high levels of correct assignment.

In a study by Farid et al., (1999) between $90.2 \%$ and $99.7 \%$ of the 1000 simulated individuals were correctly assigned. These authors found the assignment test to be a powerful method for identifying the population membership of individuals in livestock. This was also supported by Buchanan et al. (1994). As expected this study also indicated that the number of individuals from each breed that was mis-assigned to other breeds was inversely related to the genetic distances between the breeds. Correct classification of a high number of individuals, even if these were genetically close to each other, makes the assignment test with the panel of microsatellite markers used in this study a useful tool to determine the purity of breeds. This study will also contribute to parentage verification of pure and cross-bred animals.

## Indigenous and Locally developed breeds

Limited genetic information is available on the indigenous and locally developed breeds using microsatellite markers. Most of the results obtained in this study are the first attempt to genetically characterize these breeds.

A total of 13 breeds using the 24 optimized microsatellite markers were genotyped. Table 9 indicates that there were some breeds with several loci that deviated significantly ( P < 0.05) from HWE. INRA63, OARFCB20, CSRD247, ILSTS087, RM004, MAF65 and INRA23 had most deviations from HWE. MCM527 deviated from HWE in all breeds and should be disregarded in population studies. All loci showed either deficiency or excess of heterozygotes. Most of the loci showed high levels of excess heterozygotes.

Eleven out of 13 breeds had HWE deviations at loci OARFCB20 and RM004. Forty six percent of the breeds studied deviated from HWE. BM1818, MAF214, SRCRSP5, TGLA126, and ILSTS002 were in HWE across most of the breeds. There was no breed that deviated from HWE across all loci. Ideal HWE breeds do not actually occur in nature owing to various factors, which can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure. Since the sheep breeds do not represent natural populations, and because of direct selection of domesticated livestock, it was no surprise that the breeds deviated from HWE at most loci. Three out of 13 breeds had deviations from HWE at only 12 loci.

In a study by Mugai (2002), six of the markers were similar to those used in the present study (TGLA53, OARFCB20, MCM527, MAF214, MAF65 and SRCRSP5). The South African fattailed Pedi, the fat-rumped Blackhead Persian and the Damara, a Namibian sheep breed was included in both studies. The Pedi showed adherence to HWE at loci SRCRSP5 and the Damara at MAF 214.

The linkage disequilibrium tests within each of the 13 breeds were performed for the 278 pairwise combinations over the 24 loci. On average, $3.26 \%$ of the locus pairs were in linkage disequilibrium within the 13 breeds assuming a $1 \%$ type 1 error rate $(\mathrm{P}<0.01)$. Assuming a $5 \%$ type 1 error rate $(\mathrm{P}<0.05)$ between $12.32 \%$ of the loci were in linkage disequilibrium (Table 10). From the results obtained with the Merino genotypes using the same marker set, the percentage loci in linkage disequilibrium was $50 \%$. Mugai (2002) indicated on average $4.11 \%$ of locus pairs that were in linkage dise quilibrium using 9 loci.

The MNA and heterozygosities calculated, indicate the genetic variability within the indigenous and locally developed sheep breeds in this study (Table 11). The standard deviation of the MNA varied between 1.69 and 3.33. A total of 303 alleles were observed in the 13 breeds. The most polymorphic markers were INRA63 and BM1818 with 18 and 17 alleles respectively. The least polymorphic loci were MGTG4B and RM004 with 8 alleles respectively. Allele frequencies across all loci ranged from 0.122 to 0.912 (Appendix 2). A mean PPL of $85 \%$ was obtained when compared with the Merino genotypes using the same number of microsatellites, the indigenous and locally developed breeds had a total of 36 alleles more. This result can be due to more breeds tested and also a wide range of alleles that occur in the indigenous breeds. It was interesting to note that although the indigenous and locally developed breeds showed the highest number of alleles, the PPL was much lower than that observed in the Merino genotypes. A possible explanation for this result can be that the microsatellite markers were developed mainly in the European countries and tested on wool breeds. According to their selection, these breeds indicated higher polymorphism.

Table 11: Sample size, mean number of alleles (MNA), percentage of polymorphic loci (PPL), expected heterozygosity $\left(\mathrm{H}_{\mathrm{E}}\right)$ and observed heterozygosity $(\mathrm{Ho})$

| Breed | n | MNA | PPL | $\mathrm{H}_{\mathrm{E}} \pm$ s.e | $\mathrm{H}_{\mathrm{o}} \pm$ s.e |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 34 | 4.64 | 83 | $0.581 \pm 0.051$ | $0.490 \pm 0.021$ |
| 2 | 30 | 6.71 | 100 | $0.676 \pm 0.032$ | $0.541 \pm 0.019$ |
| 3 | 40 | 7.04 | 100 | $0.668 \pm 0.026$ | $0.517 \pm 0.018$ |
| 4 | 35 | 4.33 | 96 | $0.523 \pm 0.050$ | $0.481 \pm 0.018$ |
| 5 | 32 | 4.59 | 92 | $0.590 \pm 0.039$ | $0.418 \pm 0.022$ |
| 6 | 19 | 5.10 | 75 | $0.553 \pm 0.067$ | $0.423 \pm 0.027$ |
| 7 | 33 | 5.94 | 67 | $0.534 \pm 0.050$ | $0.406 \pm 0.022$ |
| 8 | 27 | 4.73 | 92 | $0.544 \pm 0.037$ | $0.334 \pm 0.021$ |
| 9 | 26 | 4.00 | 67 | $0.480 \pm 0.054$ | $0.353 \pm 0.026$ |
| 10 | 35 | 5.38 | 100 | $0.655 \pm 0.024$ | $0.558 \pm 0.020$ |
| 11 | 27 | 6.50 | 100 | $0.698 \pm 0.032$ | $0.588 \pm 0.020$ |
| 12 | 34 | 4.09 | 42 | $0.490 \pm 0.065$ | $0.368 \pm 0.028$ |
| 13 | 34 | 6.05 | 92 | $0.717 \pm 0.035$ | $0.491 \pm 0.019$ |
| Mean |  | 5.32 | 85 | $0.593 \pm 0.043$ | $0.459 \pm 0.022$ |

1-Damara; 2Karakul; 3-Pedi; 4-Blinkhaar Ronderib Afrikaner; 5-Van Rooy; 6-Blackhead Persian; 7-Blackhead Speckled Persian; 8-Redhead Persian; 9-Redhead Speckled Persian; 10-Zulu; 11-Swazi; 12 -Namaqua Afrikaner; 13-Dorper

Private alleles were observed in the following breeds: Damara (2), Karakul (11), Pedi (9), Ronderib Afrikaner (4), Blackhead Persian (7), Blackhead Speckled Persian(5), Redhead Persian (2), Zulu (3), Swazi (6) and Dorper (1). Some of the private alleles observed in the Ronderib Afrikaner were in high frequencies (0.543). The gene frequencies, across the indigenous and locally developed breeds, observed for the private alleles ranged from 0.014 to 0.543 (Appendix 2). For the Karakul there were many distinct alleles that can be used as possible breed markers. For example OARFCB20, ETH225, MCM527, TGLA57, CSRD247, ILSTS087, TGLA126 and INRA231 are markers that can distinguish between the Karakul and Ronderib Afrikaner. Alternate alleles OARFCB20, TGLA57, ILSTS087, ETH10, INRA23 and BM1824 can be regarded as breed specific loci for Swazi sheep. Alleles within CSSM36, ILSTS087 and ILSTS002 are specific for Zulu sheep. Private alleles observed within the Persian breeds indicate that the Blackhead and Redhead Persians can be distinguished. Only one private allele was observed in the Karakul by Mugai (2002). In this study, although in low frequencies at some loci, 11 private alleles were observed for the Karakul. This can support the genetic difference of the Karakul breed from the other indigenous and locally developed breeds.

The mean number of alleles ranged from 4.00 in the Redhead Speckled Persian to 7.04 in the Pedi. In general the mean number of alleles was high for sheep as was expected since the markers were specially selected for being highly polymorphic (Table 11). The Redhead Speckled Persian and Namaqua Afrikaner were the least diverse populations with a MNA of 4.00 and 4.09 respectively. The most diverse populations were observed in the Karakul and the Pedi with a MNA of 6.71 and 7.04 respectively. The MNA of the Damara, Pedi, Blackhead Persian and Karakul compared favorably to the values obtained by Mugai (2002).

Sargent et al. (1999) reported a low PPL (33\%) for the Namaqua Afrikaner. In this study a PPL of $42 \%$ was obtained which indicates that both blood proteins and microsatellite markers confirm the Namaqua Afrikaner as the least polymorphic breed in the study.

The $\mathrm{H}_{\mathrm{E}}$ ranged from $48 \%$ in Redhead Speckled Persian to $71 \%$ in the Dorper. $\mathrm{H}_{\mathrm{O}}$ ranged from 33\% in Redhead Persian to 58\% in Swazi sheep (Table 11). The average $\mathrm{H}_{\mathrm{E}}(59 \%)$ was higher in all breeds when compared to the $\mathrm{H}_{\mathrm{O}}(45 \%)$. The overall Ho
values were much lower than that reported in other studies. Mugai, (2002) reported Ho values of $0.689,0.622,0.628$ and 0.698 for the Damara, Pedi, Blackhead Persian and Karakul respectively. In this study Ho values obtained for the same breeds were $0.490,0.517,0.423$ and 0.541 respectively. The lower values can possibly be explained by the larger set of microsatellite markers used in the present study. Between the Damara and Pedi, both fat-tailed sheep, the Pedi showed more genetic variability.

The matrix for genetic distance estimates is shown in Table 12. The Letelle genotype is known to be a developed fine wool genotype was included in this part of the study as a reference. The genetic distance estimates ranged from 0.223 (Blackhead Speckled Persian and Redhead Speckled Persian) to 1.103 (Ronderib Afrikaner and Namaqua Afrikaner).

The Speckled Persian varieties show a close relationship with each other. This supports the development and selection of the Redhead Speckled Persian which can be the result of a recessive colour present in the Blackhead Persian from which it was developed. The Namaqua Afrikaner and Ronderib Afrikaner, both fat-tailed breeds, show a genetic distance estimate of 1.103 which indicates that these breeds are relatively distant from each other.

Between the Bla ckhead Persian and the Dorper, a genetic distance estimate of 0.298 was observed. This estimate is relatively low and can indicate that the present day Dorper is genetically closer to the Dorset Horn than the Blackhead Persian, the two parent breeds. A genetic distance estimate of 0.182 was observed between the Zulu and Swazi. This indicates a close relationship between these two breeds and can possibly suggest a common ancestry.

The genetic distance estimates observed between the fattailed breeds (Damara, Karakul, Pedi, Ronderib Afrikaner, Van Rooy, Zulu and Namaqua Afrikaner) were relatively high ( $0.610,0.579,0.713,0.457,0.440$ and 0.939 ) confirming genetic differences between these breeds. The developed breeds that include the Dorper and Van Rooy indicated a genetic distance estimate of 0.398 .

A smaller genetic distance estimate (0.170) was reported by Mugai (2002) for the Damara and Pedi as compared to the estimate of 0.579 obtained in this study. This study indicates a well-differentiated difference between the two breeds. Between the

Damara (fat-tailed) and the Blackhead Persian (fat-rumped) a genetic distance estimate of 0.368 was reported and between the Pedi, also a fat-tailed breed, and the Blackhead Persian an estimate of 0449 (Mugai, 2002). These estimates were slightly smaller than that observed in this study of 0.461 and 0.611 respectively. These estimates clearly indicate that genetic differences between fat-tailed and fat-rumped sheep exist. A high genetic distance estimate was observed between the Karakul and Damara (0.610) in this study. It is clear with these results that the Karakul can be regarded as genetically different from other indigenous fat-tailed breeds.

The Van Rooy also showed a close genetic distance with the Speckled Persian breeds ( 0.398 and 0.469 ) which can be a result of Speckled Persian rams being crossed with Van Rooy ewes to improve the gene pool (Campbell, 1995). A high genetic distance estimate (0.692) was observed between the Blinkhaar Ronderib Afrikaner and Van Rooy. This can indicate that the Van Rooy is more of the Rambouillet type from which it was developed although the Blinkhaar Ronderib Afrikaner contributed to its development.

Table 12: Genetic identity (above diagonal) and genetic distances (below diagonal) between indigenous and locally developed breeds

| POP | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |  |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 | $* * * *$ | 0.544 | 0.561 | 0.490 | 0.633 | 0.631 | 0.632 | 0.631 | 0.568 | 0.644 | 0.688 | 0.391 | 0.648 | 0.418 |  |
| 2 | 0.610 | $* * * *$ | 0.583 | 0.551 | 0.575 | 0.548 | 0.421 | 0.507 | 0.387 | 0.601 | 0.660 | 0.365 | 0.603 | 0.468 |  |
| 3 | 0.579 | 0.539 | $* * * *$ | 0.439 | 0.550 | 0.543 | 0.529 | 0.591 | 0.511 | 0.611 | 0.626 | 0.397 | 0.599 | 0.410 |  |
| 4 | 0.713 | 0.596 | 0.824 | $* * * *$ | 0.501 | 0.528 | 0.426 | 0.396 | 0.412 | 0.560 | 0.553 | 0.332 | 0.501 | 0.444 |  |
| 5 | 0.457 | 0.554 | 0.599 | 0.692 | $* * * *$ | 0.677 | 0.672 | 0.692 | 0.626 | 0.624 | 0.621 | 0.554 | 0.672 | 0.557 |  |
| 6 | 0.461 | 0.601 | 0.611 | 0.639 | 0.390 | $* * * *$ | 0.721 | 0.732 | 0.702 | 0.582 | 0.593 | 0.495 | 0.743 | 0.423 |  |
| 7 | 0.459 | 0.865 | 0.638 | 0.853 | 0.398 | 0.328 | $* * * *$ | 0.754 | 0.800 | 0.620 | 0.579 | 0.632 | 0.667 | 0.353 |  |
| 8 | 0.461 | 0.680 | 0.526 | 0.928 | 0.369 | 0.312 | 0.283 | $* * * *$ | 0.622 | 0.649 | 0.635 | 0.507 | 0.698 | 0.411 |  |
| 9 | 0.566 | 0.949 | 0.671 | 0.888 | 0.469 | 0.354 | 0.223 | 0.476 | $* * * *$ | 0.602 | 0.561 | 0.553 | 0.681 | 0.468 |  |
| 10 | 0.440 | 0.509 | 0.492 | 0.580 | 0.471 | 0.541 | 0.477 | 0.432 | 0.508 | $* * * *$ | 0.834 | 0.559 | 0.722 | 0.510 |  |
| 11 | 0.374 | 0.415 | 0.469 | 0.593 | 0.477 | 0.522 | 0.547 | 0.454 | 0.578 | 0.182 | $* * * *$ | 0.511 | 0.712 | 0.465 |  |
| 12 | 0.939 | 1.009 | 0.924 | $\mathbf{1 . 1 0 3}$ | 0.591 | 0.703 | 0.459 | 0.679 | 0.592 | 0.582 | 0.671 | $* * * *$ | 0.605 | 0.362 |  |
| 13 | 0.434 | 0.506 | 0.513 | 0.691 | 0.398 | 0.297 | 0.405 | 0.360 | 0.385 | 0.325 | 0.340 | 0.503 | $* * * *$ | 0.528 |  |
| 14 | 0.871 | 0.760 | 0.892 | 0.812 | 0.585 | 0.863 | 1.042 | 0.890 | 0.759 | 0.674 | 0.766 | 1.016 | 0.639 | $* * * *$ |  |

1-Damara; 2-Karakul; 3-Pedi; 4-Blinkhaar Ronderib Afrikaner; 5-Van Rooy; 6-Blackhead Persian; 7-Blackead Speckled Persian; 8-Redhead Persian; 9-Redhead Speckled Persian; 10-Zulu; 11 -Swazi; 12-Namaqua Afrikaner; 13-Dorper; 14-Letelle

The overall Fst estimate for all loci was 0.341 . The level of breed differentiation was low indicating that $48 \%$ of the total genetic variation was explained by breed differences (Table 13). The least genetically differentiated population pairs were Karakul and Pedi with Fst values of 0.010. The highest genetic differentiation (0.437) was observed between the Ronderib Afrikaner and Namaqua Afrikaner. The Namaqua Afrikaner was found to be the most genetically different from the other breeds. The genetic differentiation observed among the breeds was highly significant ( $\mathrm{P}<0.001$ ) for all breeds indicating that there were significant levels ( $\mathrm{P}<0.05$ ) of genetic differentiation between the breeds. These results are thus supported by the other estimates obtained in the study. The Rst calculations produced similar results to the Fst values and are not presented.

Table 13: Genetic differentiation (Fst - below diagonal) and gene flow (Nm - above diagonal) of indigenous and locally developed breeds

| $* * *$ | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ | $\mathbf{1 3}$ | $\mathbf{1 4}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | *** | 1.228 | 1.244 | 0.903 | 1.648 | 1.235 | 0.735 | 1.511 | 0.863 | 1.339 | 1.597 | 0.390 | 2.025 | 0.802 |
| 2 | 0.169 | $* * *$ | 2.276 | 1.519 | 1.382 | 1.068 | 0.577 | 1.143 | 0.560 | 2.255 | 2.906 | 0.354 | 1.773 | 1.028 |
| 3 | 0.167 | 0.010 | $* * *$ | 1.208 | 1.298 | 1.050 | 0.617 | 1.294 | 0.609 | 2.257 | 2.592 | 0.358 | 1.711 | 1.001 |
| 4 | 0.217 | 0.141 | 0.171 | $* * *$ | 0.940 | 0.769 | 0.487 | 0.725 | 0.518 | 1.527 | 1.597 | 0.322 | 1.077 | 0.768 |
| 5 | 0.132 | 0.153 | 0.162 | 0.210 | $* * *$ | 1.569 | 0.809 | 1.913 | 0.848 | 1.378 | 1.534 | 0.413 | 2.383 | 1.000 |
| 6 | 0.168 | 0.190 | 0.192 | 0.245 | 0.138 | $* * *$ | 1.231 | 1.720 | 0.982 | 1.044 | 1.146 | 0.457 | 1.938 | 0.840 |
| 7 | 0.254 | 0.302 | 0.289 | 0.339 | 0.236 | 0.169 | $* * *$ | 0.801 | 0.902 | 0.641 | 0.639 | 0.654 | 0.757 | 0.491 |
| 8 | 0.142 | 0.180 | 0.162 | 0.257 | 0.116 | 0.127 | 0.238 | $* * *$ | 0.686 | 1.335 | 1.462 | 0.376 | 2.339 | 0.783 |
| 9 | 0.225 | 0.309 | 0.291 | 0.325 | 0.228 | 0.203 | 0.217 | 0.267 | $* * *$ | 0.674 | 0.645 | 0.481 | 0.823 | 0.563 |
| 10 | 0.157 | 0.010 | 0.091 | 0.141 | 0.154 | 0.193 | 0.280 | 0.158 | 0.271 | $* * *$ | 5.051 | 0.386 | 1.948 | 1.080 |
| 11 | 0.135 | 0.079 | 0.088 | 0.135 | 0.140 | 0.179 | 0.281 | 0.146 | 0.279 | 0.047 | $* * *$ | 0.385 | 2.277 | 1.091 |
| 12 | 0.391 | 0.414 | 0.411 | $\mathbf{0 . 4 3 7}$ | 0.377 | 0.354 | 0.277 | 0.399 | 0.342 | 0.393 | 0.394 | $* * *$ | 0.415 | 0.323 |
| 13 | 0.101 | 0.124 | 0.128 | 0.188 | 0.095 | 0.114 | 0.248 | 0.097 | 0.233 | 0.114 | 0.101 | 0.376 | $* * *$ | 1.148 |

1-Damara; 2-Karakul; 3-Pedi; 4-Ronderib Afrikaner; 5-Van Rooy; 6Blackhead Persian; 7-Blackead Speckled Persian; 8Redhead Persian; 9-Redhead Speckled Persian; 10-Zulu; 11-Swazi; 12-Namaqua Afrikaner; 13-Dorper; 14 -Letelle

The gene flow (Nm) (Table 13) was the lowest between the Blinkhaar Ronderib Afrikaner and the Namaqua Afrikaner (0.322) while the highest value of 5.051 was obtained between the Zulu and Swazi . The 5.0 migrants per generation observed in this study correspond to the genetic distance values and other measures obtained in this study.

The Damara breed is indigenous to Namibia. It was speculated that the KhoiKhoi people migrated with sheep southwards. The sheep were mostly Afrikaner types. If geographics are considered, and the biology is aken into account, it is highly unlikely that the Damara and Afrikaner type breeds mixed. This can be verified with the high genetic distance estimates as well as the low gene flow values determined in this study.

According to the breed descriptions of the Afrikaner sheep (Ramsay et al., 2001), the Blinkhaar is a variety of the Afrikaner type sheep. Both the distance and gene flow values indicate that the Blinkhaar Ronderib and Namaqua Afrikaner are genetically different breeds. This result was unexpected as a closer relationship was thought to exist.

It is evident through the genetic distance estimates and the gene flow values that the Zulu and Swazi sheep are genetically very similar. This can indicate that from a geographic viewpoint no effective barriers were present to isolate the two breeds. From the results the Pedi seems to be genetically different from most of the indigenous breeds, especially the Afrikaner types. A close similarity was observed between the Zulu and Swazi. These breeds are mainly spread throughout the eastern part of the country.

The bootstrap values in the neighbour-joining tree (Figure 11) were higher than that of the UPGMA tree (Figure 12). The neighbour-joining tree grouped the Persian breeds in the same cluster with the Dorper, Van Rooy, Damara and Namaqua Afrikaner. The Pedi formed a separate cluster. A third cluster was observed for the Zulu, Swazi, Karakul and Ronderib Afrikaner. This cluster is quite unexpected, although the bootstrap values are high. It was expected that the Zulu and Swazi would cluster together as was observed in the genetic distance estimates. A conservation programme for the Persian breeds should not include them as separate breeds but rather the group as a whole.

The UPGMA tree showed a very similar topology as to the neighbour-joining tree. The four Persian breeds were however spread between other breeds, namely the Zulu, Pedi, Dorper and Van Rooy. The tree again shows the Karakul and Ronderib Afrikaner clustered together. In this case the Blackhead Persian clustered on a separate trunk. When both the trees are taken into consideration, the topology of the
neighbour-joining tree better explains the phylogenetic relationships of the indigenous and locally developed breeds.


Figure 11: Unrooted Neighbour-joining tree representing the genetic relationships between the indigenous and locally developed breeds.


Figure 12: Unrooted UPGMA tree representing the genetic relationships between the indigenous and locally developed breeds.

The GENECLASS programme for the assignment of individuals to populations using the Bayesian method gave the best results with $94 \%$ of the individuals (372 individuals of 395) being assigned to the correct source population (Appendix 3). With the assignment of individuals to the populations a $\mathrm{P}<0.05$ was considered. The breeds with the highest level of correct assignment were the Damara, Karakul, Ronderib Afrikaner, Swazi and Dorper with $100 \%$ of the individuals being correctly assigned. The results indicated that from the Pedi, Van Rooy, Red Speckled and Black Speckled Persians individuals were assigned with the Namaqua Afrikaner. In the Blackhead Speckled Persian 4 individuals were assigned to the Redhead Speckled Persian, 1 individual assigned with the Redhead Persian and 3 with the Blackhead Persian. In the Blackhead Persian, 1 individual assigned with the Blackhead Speckled Persian and 1 individual with the Redhead Speckled Persian. In the redhead Persian 1 individual assigned with the Blackhead Persian. One individual in the Zulu assigned with Swazi. For the Namaqua Afrikaner, 1 individual assigned with the Redhead Speckled Persian. This part of the study also indicated that the number of individuals from each breed that was mis-assigned to other breeds was inversely related to the genetic distances between the breeds.

The results indicate that the different Persians should not be regarded as different breeds, but rather as different types. Most of the mis-assignment of individuals was within the Persians. The assignment test further indicates possible influence of Persian in the Namaqua Afrikaner and in reverse as well. This result is unexpected. The assignment test further supports the close relationship of the Zulu and Swazi and that possible introgression is evident from the Zulu side. The Namaqua Afrikaner assignment to the Pedi is unexpected.

## Conclusions



## CHAPTER FOUR

## CONCLUSIONS

Traditionally, classifications of breeds were based on phenotypic traits. In some cases, recent genetic studies have found differences in the structure proposed. In sheep, one has to bear in mind that morphological cha nges (eg. horns or fleece colour) were not the result of adaptations to the environment, but have a social significance and thus may not be indicative of the genetic relationships. In recent years microsatellites have proved to be very useful for the determination of genetic relationships among populations. Comparative studies between microsatellites and protein markers have highlighted the advantages of the former.

The seven Merino genotypes studied provided interesting and sometimes unexpected results. The objective was achieved and the results supported the development of the breeds. The Merino genotypes have more within breed variation than between breed variation. The SA Mutton Merino showed the highest heterozygosity level, while the Afrino had below average heterozygosity. The high genetic distance between the SA Merino and the Letelle indicates separate development, while the close similarity of the SA Mutton and the Dohne Merino indicates high gene flow and common ancestors. It was not possible to link any of the microsatellite markers to wool or meat types. The study also confirmed higher genetic variability in the SA Merino when compared to other breeds, but lower variability when compared to studies conducted in Europe. The panel of microsatellite markers used in this part of the study was a useful tool to determine the purity of the breeds and can contribute to the integrity of sheep stud breeding in South Africa.

The third objective of the study was also reached. This was a first attempt to genetically characterize all the indigenous and locally developed sheep breeds from Southern Africa and included 13 breeds widely spread over the region. It was evident from the results that little genetic differentiation exists between the four Persian breeds. For conservation purposes, these should be regarded as different types or varieties and not as separate breeds. The large genetic distance between the Namaqua and Blinkhaar Ronderib Afrikaner was unexpected. These two breeds should be
conserved as they are genetically distinct breeds. The close relationship between Zulu and Swazi indicated that no geographic barriers separated these breeds. Although phenotypically very different, the breeds have more in common genetically.

The results obtained from this study with the 24 optimized microsatellite markers and the applied statistical analysis can now contribute to the establishment of routine DNA typing service within the ARC- Animal Improvement Institute to the advantage of the sheep industry in South Africa and for forensic application to the South African Police Services.

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## CHAPTER FIVE

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## Appendices



## CHAPTER SIX

## APPENDIX 1

## BREED DESCRIPTIONS

## DAMARA

This fat-tailed sheep arrived in South Africa between 200 and 400 AD (Ramsay et al, 2001). The Damara, Herero, Namaqua and the Kam Karrin Hottentot tribes farmed with what has become the Damara sheep of today. The name is derived from the Damara area of Namibia where the largest number of animals is found. These sheep are predominantly brown but a number of colour varieties occur. The Damara is covered with short brown or white hair. During winter, the hair is much thicker than during the summer months.

This indigenous sheep of Namibia is limited to its homeland and in the far north of Damaraland, and especially in the Kaokoveld from where they are found right to the border of Angola.


Figure 13: Damara

The Damara is a functional, efficient sheep. The Damara breed can be regarded as the ultimate example of an easy care sheep breed well adapted to the hot dry conditions of Southern Africa. They breed under harsh conditions and can survive where water, grazing and shelter are restricted. The Damara is tolerant to most sheep diseases as well as most external and internal parasites (Ramsay et al, 2001). This breedcan tolerate extreme climatic conditions and still cover great distances in search of food and water. The ewes are smaller as compared to the rams. Rams are usually horned, heavy and masculine. Ewes are light, polled and feminine. Damara ewes are very protective of their lambs and will fight off predators. This is also proof that the Damara was formed by nature and not by man. High fertility is one of the best charcteristics of the ewes as well as excellent mothering ability. The Damara is on of the sheep breeds in the world that did not undergo a colour pattern genetic bottleneck. The only stipulation is that the skin should be thick, mobile and have a good dark pigmentation. Damara skins are extremely valuable and are used to manufacture light-weight leather articles such as gloves and light-weight jackets (SA Livestock Breeding, 1998).

## KARAKUL

Archaelogical evidence indicates that the Karakul existed as early as 1400B.C. (Plug \& Badenhorst, 2001). It is one of the oldest domesticated sheep breeds in the world. The Karakul derived its name from a lake in the Turkestan, Russia, Karakul. However the breed probably originated in Arabia, and spread to the Russian steppes, Middle East, Germany and other countries, including South Africa. In 1916, Alan Thompson brought the first three Karakul rams and 84 ewes to Grootfontein College of Agriculture in Middelburg, Eastern Cape. The Karakul is a large, sturdy, prolific and mobile animal, with a long, slender head and an arched nose. The harsh conditions under which they evolved has given them strong and lasting teeth, a key to their longevity. The Karakul adapts well to the arid regions of South Africa and Namibia. They are resistant to internal parasites and foot rot. They respond well to good feed and care, are excellent foragers and will go through a season of scant food where other sheep would not be able to survive. They can withstand extreme heat and cold but should have access to dry cover and be kept out of marshy pastures. Karakul ewes are very protective and attentive mothers, resulting in a high lamb survival rate. The breed possesses a strong flocking instinct.

The Karakul differs in conformation from many other breeds. They are of the fat broad-tailed type of sheep and are medium in size. In their large tail is stored fat, a source of nourishment when grazing is scarce. The Karakul is distinguished by its coloured black fleece which may turn grey with maturation. Other colour shades do occur. They are mainly bred for fur pelt production. South Africa markets half of all Karakul pelts in the world (Terblanche, 1978; Farmers Weekly, April 3, 1998). The breed produces a light fleece with strong fibers. Namibian carpet weavers use Karakul wool for their products. This breed is also a source of by-products, including milk and tallow.

## PEDI

The fat-tailed Pedi sheep arrived in South Africa between 200 and 400 AD with the Bapedi people who migrated southwards into the Northern Province and settled in the area south of the Soutpansberg (Ramsay et al, 2001). The Pedi is a small framed naturally polled fat-tailed sheep with a flat, shallow body and long legs. Their long strong legs allow them to walk long distances in search of food. The fat tail is usually long and straight. Variations in the shape of the tail do occur; some tails turn inwards while others turn outwards. The coat colours vary from uniform brown through white with a red to brown head to a variety of black and white patterns. The most common colour is white with a red-brown head. This was due to selection for this colour in the foundation flock at the Stellenbosch Research Station, Limpopo Province. Lambs are born with smooth hair, but after three months it becomes a wool-like coat that sheds after a few years. The adult sheep have a smooth coat becoming woolly after the winter to protect the sheep against the cold weather. Their noses are uncinated with a well-developed jaw. Pedi ewes have excellent mothering abilities and will defend their young at every expense. They are also able to produce twins without difficulty. The Pedi is a hardy, heat and parasite-tolerant sheep for the semi arid bushveld.


Figure 14: Pedi

## BLINKHAAR RONDERIB AFRIKANER

The Afrikaner sheep had their origin in the Middle East and North East Africa. They migrated southward with the Khoikhoin people, moving into South Africa between 400 and 600 AD and migrating down the west coast to the southern tip of the continent. At that stage, the sheep were multi-coloured and were commonly known as Cape fat-tailed sheep. In the $18^{\text {th }}$ century, Cape Dutch farmers began selecting against coloured coats. These sheep formed the basis of the present day Afrikaner. The name " Ronderib" is derived from the fact that the the rib is oval in shape. The Blinkhaar Ronderib Afrikaner is a typical fat-tailed arid area sheep with the ability to store energy in its tail. They have a white creamy covering of hair and wool. A soft, silky white downy wool is found on the undercovering of the skin, while the fleece contains soft, long hair. The shiny hair and round-ribbed (blinkhaar ronderib) appearance is typical of the breed and strict standards are maintained by the breed society. Amber coloured horns are found on the rams, while ewes may have smaller horns. The neck is markedly thin and the breed has typical hanging ears. As an arid breed, the legs are long and thin, the rump sloping and the body is deep with an oval shaped centrpiece. This conformation allows the animal to walk long distances in search of grazing and water. The Blinkhaar Ronderib Afrikaner contributed to the development of the Vandor and Van Rooy breeds (Ramsay et al, 2001).


Figure 15: Blinkhaar Ronderib Afrikaner

## VAN ROOY

In 1906, near the Bethulie district Senator J.C. van Rooy began experiments to develop a sheep bred for slaughter lamb production. The requirements he set for this breed, were threefold:

1. The breed had to be strong and hardy to cope with regular droughts.
2. It had to be fertile in order to maintain a high percentage of production.
3. It had to have an excellent conformation.

With this in mind, Blinkhaar Afrikaner rams and Rambouillet ewes were mated. Selection for size and hardiness were the main criteria. The Van Rooy is a large, white fat-tailed mutton breed with wool and bristly hair to protect against the cold. The tail is characteristically fatrumped which also classifies this breed as a fat rumped breed. Van Rooy sheep are still found in the arid areas where survival and reproduction on natural grazing are essential for the econmic production of meat. Some farmers take advantage of the hardiness of the Van Rooy ewe and crossbreed with the Dorper, and Dohne Merino to produce a heavier slaughter lamb (Campbell, 1995).

The milking ability of the Van Rooy ewe coupled with her ability to utilise natural grazing in adverse conditions allow for maximum production in the arid areas.


Figure 16: Van Rooy

## NGUNI SHEEP

The Nguni sheep of Zululand and Swaziland migrated to South Africa with the Nguni people between 200 and 400 AD down the eastern coast to the areas where they are found today. Several routes were taken by the Iron Age people in their migration to the south. One group came down the east coast into Natal and then dispersed further south. This dispersal was limited by the growing conditions of their crops, such as millet, which could not be grown on the cold plateau of the Highveld. These people brought sheep and cattle with them and it is these which are thought to be ancestors of the present Nguni breeds.

The Nguni sheep are divided into three groups - the Swazi sheep, the Zulu sheep and the Pedi. The Zulu sheep have a mixed appearance in that their tails can be either thin or fat, they have different colours and sizes and they can have a coat of either wool or hair. This variation seems to point to recently crossbred sheep but in fact is due to a broad ance stral gene pool. This broad gene pool can give rise to a varied physical appearance but also supplies the genes necessary for the animals' adaptation to different and sometimes challenging conditions. This breed can, in fact, survive and flourish where other sheep breeds die. Generally, this breed has a black, brown or reddish brown coat that is sometimes pied. The tail is fat, long and carrot shaped. However, sheep do occur which have a long thin tail which contain very little fat. The ears, which are short and narrow, are sometimes very small mouse ears. They tend to be more 'woolly' than breeds such as the Pedi and Damara. The numbers of this breed are declining rapidly due to replacement by imported breeds and to the stock theft which is prevalent in KwaZulu-Natal (Ramsay et al, 2001).


Figure 17: Nguni

## BLACKHEAD PERSIAN

The ancestors of the Blackhead Persian arrived in South Africa by chance in 1869. According to Campbell \& Hofmeyr (1972), a vessel damaged by a storm at sea carried a number of slaughter sheep. These sheep, one ram and three ewes, were taken to Wellington where the breed was further developed.

The Blackhead Persian is classified as fat-rumped breed and is one of the parent breeds of the well-known Dor per. The Persian shows some resistance against ticks and tick-borne diseases and thrive in the Uitenhage bushveld of the Eastern Cape, Savannah-veld of the Northern Cape and even in the Kalahari dunes of the North-west Cape.

The body covering consists of short, white lime-like kemp, whereas the head is covered with short, shiny black hair. These characteristics protect the sheep against ultra-violet radiation and skin cancer (Terblanche, 1978). The breed stores energy in the form of fat in its tail. They are extremely fertile and can produce lambs throughout the year. This is a hardy, adapted breed, with a relative high natural resistance against most diseases, eg. blue tongue, pulpy kidney and tribulosis (Campbell, 1995).

Persians produce strong, valuable skins known in the trade as "glovers" which are used to produce high quality leather goods. Karakul farmers use Blackhead Persian ewes to upgrade their breeding stock to ensure that the good characteristics of the breed, such as fertility, unlimited breeding season and hardiness are utilized.

## SPECKLED PERSIAN

The Speckled Persians can be black or brown with white spots covering the body. Epstein (1971) stated: "The coat colour is basically white with a second colour usually light to dark brown super-imposed. Some are roan owing to an admixture of bright brown hair. The speckled colour of the Persian seems to be the results of a recessive colour of fat-rumped sheep.

Some South African breeders have proved that by mating a Blackhead Persian or a Redhead Persian ram to white Van Rooy or Ronderib ewes, red speckled lambs can be bred.


Figure 18: Persian breeds

## NAMAQUA AFRIKANER

The original Afrikaner sheep migrated south with the Khoikhoin people and entered South Africa between 200 and 400 AD. The Nama people kept the original Namaqua Afrikaner in the harsh dry areas of the North-west Cape and southern Namibia.

Crossbreeding for a more marketacceptable carcass almost led to the extinction of this breed. On the recommendation of Drs Q P Campbell, J H Hofmeyr and J Swart, flocks were established at the Klerefontein Experimental Station and Grootfontein Small Stock Research Institute (Ramsay et al, 2001).

The Namaqua is a long-legged sheep that can walk long distances in search of food and water. They can also tolerate intense heat as well as low night temperatures. They are covered with smooth, shiny hair, and the head colour can range from black to brown to red. Most individuals of this breed have corkscrew tails that turn either right or left at the hocks. The Namaqua is a virile sheep bred that has a long life span and their production and reproduction performance recorded under extreme conditions compares favorably with oher South African breeds. By utilizing their fat reserves, the ewes are able to wean heavy lambs even under severe drought conditions. Their mothering ability is excellent, and will fiercely protect their young against predators. Ewes are prolific, mature early and have an average lambing cycle of nine months. The ewes can lamb at an early age throughout the year. The Namaqua Afrikaner carcass is narrow, with more localized fat in the tail and rump area. The skin is suited for light mass leather goods such as gloves and jackets (Farmers Weekly, March 1998).


Figure 19: Namaqua Afrikaner

## DORPER

The Dorper is the result of one of the most successful long-term livestock improvement programmes in South Africa. The breed originated from the successful crossing of a British Dorset ram and a Blackhead Pesian ewe in 1936 by the Meat Control Board at the Grootfontein College of Agriculture. Their aim was to develop a hardy mutton sheep capable of surviving, reproducing and producing fast-growing lambs off the veld in the low rainfall areas of the country (http://www.studbook.co.za).

The Dorper is large, well-conformed mutton sheep with a white or black head and a white body. The breed has a long breeding season which is not seasonally limited. They can produce lambs at almost any time of year and reach a lambing percentage of 150 per season under optimum grazing conditions. Dorper ewes produce large quantities of milk and have good mothering abilities. The breed is well adapted to a variety of climatic and grazing conditions. Originally the breed was developed for the more arid areas of South Africa, but today they are widespread throughout all provinces. The Dorper does well under various veld and feeding conditions and reacts most favorably under intensive feeding conditions.

It is an easy-care breed requiring minimum labour. Its skin covering which is a mixture of hair and wool, will drop off if not shorn to keep it tidy. The Dorper has a thick skin which is highly prized and protects the sheep under harsh climatic conditions. The Dorper skin is the most sought-after sheepskin in the world and is marketed under the name of Cape Glovers. The skin comprises of a high percentage of the income ( $20 \%$ ) of the total carcass value.


Figure 20: Dorper sheep

## THE SOUTH AFRICAN MERINO BREEDS

## HISTORY

South Africa was the first country outside Europe that stocked Merinos. The history dates back to 1789 , when the Dutch Government donatedtwo Spanish Merino rams and four ewes to Jacob Gordon, military commander in the Cape at the time, on an experimental basis.

By 1830, wool-sheep farming was well underway in the Western and Southwestern Cape. The next expansion was eastwards. The 1820 settlers played an important role in this extension and development of Merino flocks. In 1834, The Great Trek began. Within a few years the Merino had spread to all parts of the country.

From 1891, considerable numbers of Merinos of the American Vermont type were brought to South Africa. However, it was found that the Australian Merino, the Wanganella and Peppin type were best suited to improve our flocks, and large numbers of this breed were imported (http://www.studbook.co.za).

With the different types forming the basis, the South African breeders have succeeded in developing typical Merino on a par with the best of the world. These Merinos, which have developed in the course of more than 200 years, form the backbone of South Africa's Agricultural Industry.

Different types of Merino sheep were developed for different regions in South Africa. These types vary from strong wool to the finest wool and plain-bodied sheep for the drier Karoo regions to a medium developed sheep for the lucerne lands of the Western Cape, Northern, Freestate and irrigation areas. Merinos are found in great numbers in the drier Northern Cape province, on the fertile lands of the winter rainfall areas of the Western Cape and millions of Merino sheep run on the Karooveld and savanna of the Eastern Cape and Free State. Well known Merino Breeders with large top quality flocks are also found in the East Griqualand, Kwazulu Natal and most parts of Mpumalanga.

## DORMER

The Dormer sheep breed originated because there was a need to develop a breed that can adapt to the climatic and grazing conditions of a winter-rainfall region and still produce young slaughter lambs of high quality. The Dormer was developed at the Elsenburg Research Station in the Western Cape by crossing Dorset Horn rams and German Merino ewes. Further development took place in co-operation with farmers in the Western Cape Province where winter pastures are used for slaughter production.

The Dormer is a white-wool mutton sheep with a sturdy frame - developed for the climatic and grazing conditions of the Western Cape. The breed has a high fertility, long breeding season and the capability to produce multiple siblings. The body of the sheep is covered with rough white wool while the head is covered with white calcareous hairs. The resistance against worms, for which it was selected in the development of the breed, is still an important characteristic of the Dormer in the winter-rainfall. The Dormer was bred exclusive ly for meat production; slaughter lambs reach a slaughter mass of approximately 15 kg after three to four monthes. As a temperate climate breed, it is now fairly widely distributed in the Free State and Gauteng Provinces (SA Livestock Breeding).


Figure 21: Dormer

## SOUTH AFRICAN MERINO (SA MERINO)

The SA Merino is one of the most important and numerous sheep breeds in South Africa. They are widely spread throughout the country with large numbers in the Eastern Cape, Free State and Western Cape. It is a white-wool sheep breed that holds fine fleece and consists of a reasonable mutton conformation (SA Livestock Breeding, 1998). The horns of Merino rams are large and stand well away from the head. Adult rams and ewes can reach a mass of 85 and 50 kg respectively. A lamb percentage of 80 can be obtained, although extreme management could improve this statistic. The wool is of high quality and spinning count varies from very strong to ultra-fine. Adult ewes produce 5 kg of wool during a good growth year, while adult rams could produce up to $10-15 \mathrm{~kg}$ or more. The Cape is the most important wool producing area in South Africa. The Merino's production potential for fine-wool is unique. According to Dr C.A. van der Merwe, "The Merino is the only sheep in the world that can produce $10-15 \%$ of its own live mass in clean wool (Merino Sheep Breeder's Society).


Figure 22: SA Merino

## SOUTH AFRICAN MUTTON MERINO

The breed was originally known as the German Mutton Merino. The first ten ewes and a ram were imported to South Africa from Germany in 1932 by the Department of Agriculture as part of a breeding programme. Through selection for a better wool quality and conformation, the uniqueness of the South African breed was recognised in 1971 when the breed name was changed to the SA Mutton Merino.

The SA Mutton Merino is a dualpurpose breed, developed to produce a slaughter lamb at an early age, as well as good quality wool. This breed is known for its fertility. Lambing percentages of $150 \%$ and even higher can be obtained. The SA Mutton Merino is very successful in terms of growth rate. Mature ewes have an average weight of 77 kg and rams 127 kg . Ewes produce an average of 3.4 to 4.5 kg wool and rams between 4.5 and 60 kg .


Figure 23: SA Mutton Merino

The SA Mutton Merino has a renowned mothering ability and rear multiple births to high weaning masses. They also have a high milk production enabling lambs to maintain a high growth rate for early maturing and hence early marketing. The breed is an efficient feed converter and popular in feedlots. It is non-selective in its grazing habits and no trampling of pastures occurs. The breed is able to utilize low quality
roughage. Its grazing habits limit energy consumption and better utilization efficiency is maintained allowing for increased mutton and wool production.

The SA Mutton Merino is able to perform under all climatic conditions and this also contributes to the breeds popularity. It is used often in cross-breeding programmes with other wool sheep breeds for the conformation, fertility, hardiness and adaptability of the SA Mutton Merino. The breed also contributed to the development of three other breeds in South Africa namely the Dormer, Dohne Merino and Afrino. The SA Mutton Merino is found throughout Southern Africa (Terblanche, 1979).

## THE MERINO LANDSHEEP

The Merino Landsheep has existed since the $13^{\text {th }}$ and $14^{\text {th }}$ centuries in Germany. With the importation of Merinos from Spain and France in the $18^{\text {th }}$ century the dual purpose qualities of this breed was established and has since then been purely bred in the present geno- and phenotypial properties.

The first Merino Landsheep were imported into South Africa in 1956 by a few progressive farmers in the Kroonstad area. The sheep adapted very well to the climatic and vegetational conditions. Although numbers are small, Merino Land sheep are found scattered throughout the sheep areas of Gauteng, Free State, Mpumalanga, Free State and to a lesser extent in the Cape Provinces.

The Merino Land sheep is a medium to large sheep with oval to long pollard head with a typical fringe, wide and slightly drooping ears. It has an oval rib section. The hindquarter is long and wide in the pelvic region. Pleats are unacceptable. The belly wool is well developed.

With its exceptional length and depth and long strong legs, the Merino Land sheep produces a heavy carcass weight at an early age. It is a sheep with a large frame, good walking ability, good grazing ability, well adapted under both extensive and intensive conditions with a high fertility rate, good reproduction, high milk production and good wool. This breed is prepared to search for food and eat what is available - and equally at home in the Karoo and high rainfall areas.

As a result of the present economic and climatic conditions in our country, it is a notable fact that only a high productive animal will be able to maintain its identity. The Merino Land sheep with its excellent double purpose qualities is remarkably suited for meat and wool production (SA Livestock Breeding, 1998).


Figure 24: Landsheep

## LETELLE

In 1921-1922 a certain Mr. van der Walt bought 35 large smooth bodied ewes with fine wool from Mr. Dawid Nel, Bergplaas, Barkley East. These ewes were mostly the Rambouillet type. Rams with the same qualities for the intended ideal were selected. These sheep were taken to a farm in the Wepener district where the ram were permanently left with the ewes. The intention was to let nature run its course with a little help from a breeder with instinct and knowledge. Mr van der Waly applied line breeding and strict selection for the characteristics that he has chosen as his aim. From the ewes an exceptional ram, 'Masjien', was born. He can rightly be considered as the patriarch of the Letelle.

Mr van der Walt also became involved with other breeders of good quality sheep namely Frasers Ltd of Wepener and D. Koch of Koffiefontein. When this co operation failed, the ewes that Mr van der Walt wanted was added to his flock. Mr van der Walt followed his own ideas, even when it was not in accordance with the ideas that were fashionable at the time. Only after about 25 years did the results of this venture become apparent. Today the Letelle is found in large numbers in Grootfontein in the Eastern Cape (personal communication, Jacobus le Roux).

## DOHNE

The Dohne Merino is a synthetic dualpurpose Merino developed by the South African Department of Agriculture using Peppin-style Merino ewes and German Mutton Merino sires. Progeny were selected for high fertility, rapid lamb growth rate and fine merino wool. The Dohne Merino is today one of the leading wool breeds in South Africa.

Ewes are highly fertile combined with rapid lamb growth rate, making the Dohne Merino a highly efficient meat producer. Ewes can produce between 4 to 6 kg of high quality fine wool. The easy care features of the Dohne Merino include fleece rot and fly strike resistance. Dohne Merinos are adapted to a wide range of environmental and climatic conditions from intensive production systems to extensive arid rangeland.


Figure 25: Dohne Merino

## AFRINO

A request was forwarded to the National Department of Agriculture by the wool industry, in the late 1960 's, to develop a white wool breed for the extensive sheep grazing area. The breed had to comply with the following:

* Free of kemp and coloured fibers
* Hardy and well adapted to the environment of the extensive grazing areas;
* Good slaughter lamb at an early age;
* Good reproductive ability.

In 1976, it was evident that the cross consisting of $25 \%$ Merino, $25 \%$ Ronderib Afrikaner and $50 \%$ SA Mutton Merino fulfilled the requirements for the new breed. The Afrino is a dual-purpose sheep breed. The breed is a white wool mutton breed developed for slaughter lamb and wool production under extensive conditions. The Afrino produces Merino type wool, with fiber diameter ranging from 19 to 22 micron. During selection, emphasis is placed on wool quality, while care is taken not to decrease the amount of wool produced. Wool production is only $34 \%$ of its body weight and its wool is a good export product and reaches high selling prices. Afrino ewes have outstanding mothering abilities and lambing difficulties are rare. The breed is known for its high quality meat with an even fat distribution over the carcass. These sheep are found in large numbers in the northern Cape (SA Livestock Breeding, 1998).


Figure 27: Afrino

## APPENDIX 3

## Assignment of individuals: Merino genotypes



66 67 68 70 71 72 73 74 75
76

MUT001 MUT002 MUT003 MUT004 MUT005 MUT006 MUT007 MUT008 MUT009 MUT010 MUT011 MUT012 MUT013 MUT014 MUT015 MUT016 MUT017 MUT018 MUT019 MUT020 MUT021 MUT022 MUT023 MUT024 MUT025 MUT026 MUT027 MUT028 MUT029 MUT030 MUT031 MUT032 MUT033 MUT034 MUT035 LAN001 LAN002 LAN003 LANOO4 LANOO5 LAN006 LAN007 LAN008 LANOO9 LAN010 LAN011 LAN012 LAN013 LAN014 LAN015 LAN016 LAN017 LAN018 LAN019 LANO20 LAN021 LAN022 LANO23 LAN024 LANO25 LAN026 LANO27 LAN028 LAN029 LAN030 LAN031 LAN032 LAN033 LAN034 LAN035 LAN036
MUT035 ] 21|36.28| 58.57| 22.11|31.23| 45.59| 28.59| 31.36|= MUTO35] 22| 29.62| 50.91| 23.76| 26.65| 52.72| 28.57| 27.61|= MUT035 ] 21| 33.66| 50.80| 22.58| 30.79| 50.16| 27.29| 35.24|= MUT035] 23| 39.71| 54.05| 27.96| 38.91| 45.26| 32.44| 32.31|= MUT035 ] 17| $27.40|43.44| 20.66|27.43| 46.58|25.02| 31.12 \mid=$ MUT035] 20| 36.92 | $43.50|18.17| 29.71|39.94| 27.58|32.44|=$ MUT035] 18| 22.67| $45.05|17.55| 27.81|31.12| 25.78|24.79|=$ MUT035 ] 23| 39.80| 48.88| 25.89| 34.49| 50.94| 31.59| 29.40|=
MUTO35] 23| 41.12| 57.60| $24.03|32.03| 42.57|31.01| 32.11 \mid=$
MUTO35] 22| 28.86| 51.15| 22.63| 30.96| 35.96| 26.55| 29.36|=
MUT035 ] 23| $35.23|47.80| 24.84|38.18| 36.06|33.05| 34.40 \mid=$
MUTO35] 24| 47.27| 65.22| 30.61| 37.74| 56.46| 44.32| 43.56|=
MUT035 ] 22| 34.52| 54.37| 20.43| 32.18| 43.85|32.58| 34.46|= MUTO35] $22|36.83| 52.38|25.38| 37.22|42.13| 43.97 \mid$ 40.14|= MUT035] 22| 36.93| 55.24| 20.33| 35.18| 45.62| 31.36| 40.61|= MUT035 ] 21| 39.88|45.98| 22.63|27.60| 47.86| 34.00| 30.67|= MUTO35] 22| 42.11| 45.93| 28.41| 34.72| 53.37| 33.96| 34.49|= MUTO35] 20| 37.04| 52.27| 25.08| 33.30| 37.90| 33.71| 34.54|= MUT035] 21| 32.88| 48.12| 18.50| 29.78|45.88|34.65| 32.11|= MUTO35] 21| $41.69|58.07| 27.49|43.56| 52.92|40.41| 42.40 \mid=$ MUT035] 23| 42.01| 57.14| 28.36| 39.05| 45.81| 40.75| 41.94|= MUTO35 ] $22|44.02| 50.21|28.51| 31.80|41.73| 36.12|42.12|=$ MUTO35] 19| 29.93| 43.95| 20.94| 32.25| 31.03| 29.54| 29.14|= MUT035 ] 23| 35.80| 57.43| 25.33| 41.80| 54.78| 45.54| 46.75|= MUTO35] 22| 30.89| 55.63| 23.34| 33.27| 47.82| 32.54|32.34|= MUTO35] 20| 34.25|52.00| 23.24| 25.07| 35.27| 28.77| 37.81|= MUT035] 23| 37.40| $51.65|27.45| 40.86|54.43| 39.75|38.12|=$
MUT035] 23| $41.27|53.22| 27.05|35.00| 45.59|33.65| 43.56 \mid=$ MUT035 ] 21| 33.90| 49.90| 21.60| 27.18| 43.39| 30.28| 36.98|= MUTO35] $22|38.05| 52.18|26.37| 32.71|43.09| 40.50|38.21|=$ MUT035] 23| 35.62| 55.96| 26.16| 37.02| 49.71|36.01|36.20|= MUT035 ] $22|41.00| 55.84|25.34| 35.82|49.22| 41.16|35.02|=$ MUTO35] 20| 37.92| 54.68| 26.90| 34.68| 52.61| 34.70| 37.48|= MUT035] 21| 38.48| 45.51| 24.54| 23.50| 49.73| 28.30| $32.76 \mid$ LAN040
MUTO35] 24| $38.79|52.07| 20.10|32.37| 48.65|36.11| 34.74 \mid=$ LANO40] 22| $45.95|56.70| 38.44|26.70| 52.28|37.21| 41.44 \mid=$ LANO40 ] 23| 47.98| 52.36| 38.19| 32.43| $57.96|34.89| 39.09 \mid=$ LANO40] 21| 47.10| 46.28| 42.39| 23.80| 49.81| 30.63| 34.38|= LANO40 ] 19| 30.79| 36.59| 27.12| 19.37| 44.77| 21.78| 25.56|= LANO40 ] 23| 46.69| 54.17| 45.26| 24.21| 62.20| 39.82| 37.85|= LANO40 ] 20| $43.72|44.98| 39.77|28.57| 50.37|37.74| 37.62 \mid=$ LANO40] 21| 43.48| $57.57|41.73| 41.54|54.96| 41.03|41.51|$ DOH041
LANO40] $22|40.46| 46.52|33.83| 20.59|45.93| 34.12|39.61|=$ LANO40 ] 22 | 42.21| 52.15| 34.24| 27.93| 49.25| 36.73| 33.81|= LANO40] 20| $38.07|39.68| 30.63|27.16| 40.36|36.23| 32.09 \mid=$ LANO40] 22| 41.78| 42.95| 32.61| 19.66| 51.41| 28.13| 32.93|= LANO40] 20| 38.41| 38.87| 28.27| 21.79| 42.76| 30.53| 29.65|= LANO40] $24|45.48| 53.57|39.38| 24.86|53.48| 38.46|38.17|=$ LANO40] 20| 47.03| 43.44| 39.25| 22.49| 48.36| 31.67| 35.65|= LANO40] 23| 48.27| 47.24| 46.33| 26.67| 49.39| 40.66| 40.94|= LANO40] 23| 52.98| 50.98| $45.23|31.35| 53.94|41.41| 42.45 \mid=$ LANO40] 22| 38.63| 49.06| 27.33| 21.86| 39.08| 27.45| 35.03|= LANO40 ] 22| 46.17| $54.21|38.26| 23.49|49.70| 33.82|34.94|=$ LANO40] $22|41.50| 44.20|42.05| 22.70|41.16| 39.09|42.84|=$ LANO40] 21| 41.36| 46.90| 37.33| 24.75| 47.94| 32.39| 40.15|= LANO40] 22| 43.86| 47.01| 35.92| 25.25| 50.39| 30.15| 33.15|= LANO40] 20| 43.43| 47.77| 39.04| 28.79| 54.74| 35.76| 42.54|= LANO40 ] 22| 43.12| 48.25| 42.33| 28.33| 44.21| 36.73| 37.47|= LANO40 ] 20| 42.94| 54.54| 42.35| 29.50| 59.27| 34.81| 42.70|= LANO40] 19| 37.95| $52.66|30.56| 20.58|41.61| 29.70|36.27|=$ LANO40 ] 23| 41.91| $44.26|35.65| 20.73|47.14| 32.38|42.52|=$ LANO40] 22| 38.92| 55.48| $32.78|28.90| 39.49|33.11| 30.42 \mid=$ LANO40 $21|38.35| 49.24|34.00| 21.10|42.74| 28.49|38.16|=$ LANO40] 21| 38.13| 47.68| 34.96| 26.09| 42.27| 36.05| 44.81|= LANO40] 22| 46.99| 53.50| 41.19| 25.23| 52.13| 38.38| 32.94|= LANO40] 20| 35.97| 47.93| 31.27| 21.51| 42.02| 28.14| 37.71|= LANO40] 19| 41.56| 48.38| 36.69| 25.50| 47.55| 34.90| 36.77|= LANO40] 23| 44.59| 52.78| $37.29|26.97| 45.52|34.68| 40.61 \mid=$ LANO40] 19| 40.37| 52.38| $32.76|28.03| 45.47|30.49| 36.39 \mid=$ LANO40] 17| 32.78| 43.83| $26.58|26.52| 31.39|29.85| 29.13 \mid=$ LANO40 ] 18| 39.25| 43.20| 28.63| 18.96| 41.11| 27.48| 28.31|= LANO40] 20| 40.17| 46.36| 36.10| 17.62| 45.75| 29.01| 38.89|=



## Assignment of individuals: Indigenous and locally developed breeds

## num. name group

loc.|DAM034|KAR030|PED040|RON035|VAN032|SWP019|SSP033|RKP028|RSP026|ZUL035|SWA027|NAM034|DOR023|classified in

1
DAM001 [ DAM034] 19| $25.82|47.48| 42.24|61.58| 37.00|38.14| 37.21|45.76| 38.15|40.69| 36.80|30.97| 33.77 \mid=$
DAM002 [ DAM034] 18| 32.39| 48.62| 46.01| 50.21| 33.95| 44.77| 40.13| $51.14|42.05| 40.25|37.94| 40.47|39.40|=$
DAM003 [ DAM034] 17| 10.81| $29.75|27.66| 38.53|20.29| 18.58|22.80| 26.41|22.13| 23.08|20.37| 26.03|24.39|=$
DAM004 [ DAM034] 17| 15.86| 36.92| 38.79| 47.08| 32.35| 35.09| $25.50|33.65| 31.99|28.91| 27.53|33.18| 29.06 \mid=$
DAM005 [ DAM034] 14|16.01|31.43|32.72|36.01| $24.40|26.79| 26.99|29.96| 30.07|28.02| 23.10|27.94| 27.07 \mid=$
DAM006 [ DAM034] 18| 17.50| 35.56| 35.51| 42.78| $34.15|31.61| 40.87|46.26| 40.80|34.77| 31.59|39.70| 35.93 \mid=$
DAM007 [ DAM034] 17| 15.93|34.77|34.13|30.79|23.10|26.63|29.27|34.36|28.20|25.08|21.96|29.30|30.71|=
DAM008 [ DAM034] 18| 15.03| 31.26| 37.64|37.63| 28.52| 31.56| 32.94|39.23| 32.77| 29.32| 21.25|31.62|35.39|=
DAM009 [ DAM034] 18| 17.63| 40.89|35.87| 48.99|34.45|37.77|35.24|42.85|37.06|29.81|31.63|35.31|31.30|=
DAM010 [ DAM034] 16| 13.87| 30.26| $25.44|37.01| 15.47|20.61| 23.06|23.26| 24.43|23.28| 22.31|23.38| 21.11 \mid=$
DAM011 [ DAM034] 17| 16.99| 30.40| 32.51| 39.20| 31.08| $29.23|36.36| 43.75|35.28| 28.36|27.10| 34.17|31.53|=$
DAM012 [ DAM034] 15| 13.09| $26.55|20.21| 30.33|20.56| 25.69|27.45| 29.88|24.67| 18.29|17.02| 27.03|18.50|=$
DAM013 [ DAM034] 18|20.53| 34.97| 35.65| 39.67| $30.93|28.26| 31.02|36.93| 34.95|31.12| 33.42|37.14| 26.21 \mid=$
DAM014 [ DAM034] 16| 19.24| 35.00| 40.14| 42.62| $28.87|37.34| 35.46|37.16| 40.68|32.28| 30.01|37.12| 30.00 \mid=$
DAM015 [ DAM034] 18| 19.23| 40.53| 37.71| 49.38| 32.65| 29.32| $28.13|30.70| 35.76|32.54| 27.52|32.94| 30.76 \mid=$
DAM016 [ DAM034] 19| 16.20| 36.16| 32.31| 45.59| $24.52|28.49| 32.51|33.71| 32.75|32.99| 31.58|32.78| 28.39 \mid=$
DAM017 [ DAM034] 17| 16.52| 33.95| 38.20|39.97| 32.59| 36.23| $37.92|46.75| 38.71|22.55| 24.26|33.10| 30.26 \mid=$
DAM018 [ DAM034] 16| 19.51| $28.29|29.84| 41.96|27.44| 26.99|30.50| 33.41|26.85| 33.29|32.60| 34.28|27.40|=$
DAM019 [ DAM034] 16| 13.93| 35.41| 37.11| 38.91| 28.57| 28.68| 35.40| 35.79| 32.88| $26.06|23.11| 31.61|32.13|=$
DAM020 [ DAM034] 16| 13.01|31.43|32.87| 44.12| $24.09|25.65| 28.28|29.52| 27.70|28.68| 23.35|26.43| 22.59 \mid=$
DAM021 [ DAM034] 15| 13.50| 29.40| 31.47| 35.66| 25.09| $24.08|20.45| 25.22|25.15| 21.84|22.99| 26.02|21.98|=$
DAM022 [ DAM034] 15| 12.09| 33.19| 29.28| 38.45| 23.77| 31.88| 31.18| 32.03| 33.63| 17.45| 18.71|27.07|23.05|=
DAM023 [ DAM034] 17| 20.60| $44.83|47.79| 48.54|34.30| 38.51|37.44| 40.15|34.94| 37.46|32.71| 35.16|34.57|=$
DAM024 [ DAM034] 15| 19.38| 32.34|31.00| $47.05|33.02| 33.53|35.40| 36.84|32.21| 34.56|22.33| 34.37|26.85|=$
DAM025 [ DAM034] 16| 16.07|24.08| 28.91| 42.08| $21.03|23.08| 26.99|23.84| 29.91|27.34| 27.76|33.25| 22.53 \mid=$
DAM026 [ DAM034] 14| 10.78| 25.93| $23.30|37.59| 21.18|18.48| 19.55|23.90| 19.66|20.83| 17.93|24.22| 21.32 \mid=$
DAM027 [ DAM034] 16| 16.82| 35.26| 29.97| 43.88| $28.35|30.57| 30.15|34.17| 29.21|24.65| 25.62|31.16| 29.99 \mid=$
DAM028 [ DAM034] 17| 12.81|30.84|35.92| 43.81| $25.89|33.15| 29.11|36.63| 30.65|24.22| 22.37|33.22| 32.91 \mid=$
DAM029 [ DAM034] 13| 12.71|24.84| 29.20| $28.86|22.67| 21.74|20.23| 27.16|23.69| 19.36|18.78| 25.36|21.19|=$
DAM030 [ DAM034] 15| 13.00| $26.41|31.18| 34.50|27.20| 27.27|29.49| 31.63|29.68| 22.13|20.77| 28.12|28.16|=$
DAM031 [ DAM034] 15| 15.99| 28.99| 29.71| 39.19| $23.27|27.52| 24.47|27.79| 24.01|22.53| 25.43|30.71| 24.55 \mid=$
DAM032 [ DAM034] 15| 12.65| $23.35|32.88| 36.75|21.85| 30.04|25.18| 29.14|28.93| 20.58|19.99| 26.95|19.77|=$
DAM033 [ DAM034] 15| 13.35|25.26|26.31|32.91| 20.85| $25.89|24.72| 27.08|27.62| 19.62|19.43| 27.10|24.32|=$
DAM034 [ DAM034] 11| 10.15| $23.03|22.46| 23.62|14.36| 15.28|18.76| 14.64|15.81| 17.94|16.46| 17.99|14.85|=$
KAR001 [ KAR030] 21|47.58|23.88|47.06| 54.57|60.10| 48.27|55.25|62.01|53.79|49.47|46.44|44.97|44.21|=
KAR002 [ KAR030] 20| 49.00| 25.76| 41.84| 51.70| 39.61| 47.04| 43.24| $52.48|41.25| 48.34|42.63| 35.86|43.39|=$
KAR003 [ KAR030] 19| 46.51|24.30| 40.92| 50.18| $52.22|41.29| 46.64|51.15| 50.05|43.81| 36.62|44.34| 47.78 \mid=$
KAR004 [ KAR030] 20| 46.83| 26.47| 45.73| 57.93| 52.36| 42.43| 47.50| 52.66| 53.81| 51.99| 45.36| 41.96| 47.16|=
KAR005 [ KAR030] 21|56.51|27.36|43.23| 56.25|54.21|54.05|54.75|58.55|55.61|53.49|47.66|51.76|52.08|=
KAR006 [ KAR030] 21|55.19| 38.04| 55.25| 66.70| 47.04| 53.31| 52.40| 52.84| 55.50| 59.65| 49.83| 49.35| 52.35|=
KAR007 [ KAR030] 20| $50.05|19.98| 35.71|48.44| 49.39|41.47| 45.96|51.23| 46.86|47.64| 41.57|41.48| 47.74 \mid=$
KAR008 [ KAR030] 20| 46.31|23.98|38.71|52.20| 48.55| 47.61| 45.06| 53.29| 49.68|48.02| 40.31|42.67|44.22|=
KAR009 [ KAR030] 20| 42.40| 27.53|41.32| 50.98| 42.56| 37.92| 42.51|44.12|41.55|47.94|39.71|40.76|42.85|=
KAR010 [ KAR030] 22| 48.57| 28.11| 48.02| 48.84| 50.21| 43.34| 44.46| 49.88| 47.78|48.25| 42.20| 43.63| 42.01|=
KAR011 [ KAR030] 23| 50.34|22.74|46.08| 59.68| 55.42| 54.77| 51.83|59.14|56.56|49.23| 43.15|49.04|49.47|=
KAR012 [ KAR030] 22| 46.08| $27.97|47.83| 51.13|52.01| 48.75|48.54| 60.92|54.07| 49.62|48.46| 46.61|38.69|=$
KAR013 [ KAR030] 22| 57.85| $26.64|55.57| 63.58|63.42| 52.52|56.51| 64.71|61.82| 56.45|55.06| 52.86|47.77|=$
KAR014 [ KAR030] 23| 56.03| 35.27| 52.71| 69.20| 56.16| 49.62| 57.19| 55.06| 60.29|61.13| 45.71|48.23| 54.98|=
KAR015 [ KAR030] 23| $51.96|25.54| 45.14|52.53| 49.67|51.05| 56.34|58.12| 54.82|53.40| 48.68|46.50| 46.60 \mid=$
KAR016 [ KAR030] 24|51.82| $24.60|51.87| 55.30|56.19| 56.64|64.12| 64.99|63.85| 48.66|49.98| 54.61|51.95|=$
KAR017 [ KAR030] 23| 54.71| $32.39|51.38| 56.75|54.33| 56.34|53.07| 56.17|59.72| 52.92|48.10| 51.14|49.33|=$

KAR018 [ KAR030] 23| $54.41|30.73| 49.53|54.19| 51.47|44.49| 49.66|60.18| 54.81|53.69| 44.62|51.87| 46.11 \mid=$ KAR019 [ KAR030] 23|46.86|27.11|46.41|53.38|46.32| 42.93|55.96|56.82|51.27|47.09|36.94|49.73|42.93|= KAR020 [ KAR030] 23| 58.98| 34.91| 56.28| 64.72| 55.39| 55.40| 55.15| 59.69| 58.89| 52.03| 51.52| 52.08| 51.74|= KAR021 [ KAR030] 23| 54.82| 25.66| 46.00| 56.64| 53.02| 51.20| $49.35|58.12| 50.59|53.00| 50.87|47.74| 47.99 \mid=$ KAR022 [ KAR030] 23| 53.20| $22.42|45.32| 60.25|53.46| 45.95|54.47| 59.13|54.37| 48.23|42.72| 52.11|50.40|=$ KAR023 [ KAR030] 22| 42.76| 19.52| 40.50| 55.90| 42.65| 45.26| 48.97| 52.20| 49.41|44.68| 39.64|42.79|40.18|= KAR024 [ KAR030] 23| 54.60| $33.00|47.46| 68.22|54.04| 50.19|54.52| 57.25|58.35| 54.09|49.76| 49.47|55.36|=$ KAR025 [ KAR030] 23| 48.51|31.73|51.64|61.74|49.22| 50.91|51.15|58.30|56.82|52.20|45.08|45.72|51.95|= KAR026 [ KAR030] 22| 51.81|24.24| 42.86|57.49| 53.54| 42.74| 47.76| 57.51| 51.90| 52.34| 46.84| 44.24| 47.93|= KAR027 [ KAR030] 23| 45.24| 18.84| $43.35|50.55| 51.97|51.52| 54.31|58.34| 52.78|45.47| 40.97|49.40| 51.31 \mid=$ KAR028 [ KAR030] 23| 54.16| $23.18|55.98| 55.48|60.20| 53.23|59.38| 68.67|58.73| 52.51|49.79| 51.53|53.38|=$ KAR029 [ KAR030] 23| 45.47| $25.33|46.62| 58.02|44.04| 48.32|45.31| 53.12|51.01| 45.10|40.59| 42.37|44.68|=$ KAR030 [ KAR030] 23| 46.67| 29.94|51.04|51.63| 52.06| 42.88| 48.87| 52.95|46.29|49.49|50.45|48.71|49.03|= PED001 [ PED040] 15| 32.79| 32.57| 18.76|36.83| 37.26| 35.78| 37.90| $43.54|37.13| 32.78|35.00| 37.28|34.92|=$ PED002 [ PED040] 17| 39.94|38.46| 20.03| $50.73|38.62| 41.10|37.61| 44.09|35.06| 33.97|35.56| 36.63|33.80|=$ PED003 [ PED040] 18| 35.00| 32.99| 18.93| 45.55| 40.55| 39.02| 43.96| 47.23| 42.50|35.74|37.36|43.73| 36.21|= PED004[ PE PED040] 18| 39.80| 41.78| 31.49| 52.00| 47.28| 42.61| 48.35| $53.59|47.08| 41.12|43.07| 47.18|40.29|=$ PED005 [ P PED040] 19| $47.62|55.97| 29.25|64.83| 46.20|44.25| 47.52|46.05| 49.41|49.45| 48.23|43.95| 51.42 \mid=$ PED006 [ PED040] 22| 58.53| 59.33| 32.00| 69.33| 59.60| 58.13| $57.59|63.31| 52.41|52.88| 61.02|51.56| 54.07 \mid=$ PED007 [ PED040] 20|47.64|45.78|22.02|61.31|48.14|53.88|47.33|51.01|47.25|46.09|43.81|45.82|47.48|= PED008 [ PED040] 21|48.08| 46.39| 17.55| 61.35| 37.98| 44.05| 41.25| 49.65| 43.81|44.50| 44.59| 43.12| 39.33|= PED009 [ PED040] 21|44.88| 44.31| 28.63| 63.60| 50.11| 45.33| 43.19| 44.12| 44.26| 44.57|47.44|45.45|44.06|= PED010[ P PED040] 23| 53.94|57.53| $23.73|69.05| 57.67|57.81| 52.62|63.08| 49.77|54.51| 55.80|51.47| 53.73 \mid=$ PED011 [ PED040] 20| 46.14| 43.50| 17.95| 56.06| 47.40| 42.36| 43.55| 47.73| 44.71| 47.35|39.73|43.98|43.48|= PED012 [ PED040] 22| 56.41|56.73| 25.63| 60.61| 57.57| 47.16| 52.55| 53.34| 47.15|51.65|51.65|55.19|49.28|= PED013 [ PED040] 22| 55.00| 55.82| $24.20|71.65| 56.89|51.35| 49.15|52.53| 51.72|54.12| 51.57|44.20| 46.70 \mid=$ PED014 [ P PED040] 20| $41.82|45.81| 26.59|56.20| 44.37|45.08| 36.02|41.38| 41.20|37.82| 43.38|42.57| 39.77 \mid=$ PED015 [ P PED040] 22| 45.57| 47.65| 20.87| $56.93|51.78| 45.17|41.74| 45.19|43.59| 42.86|41.06| 45.71|40.37|=$ PED016 PED017 PED018 PED019 PED020 [ PED021 PED022 PED023 PED024 PED025 PED026 PED027 PED028 PED029 [ PED040] 20| 56.05| 51.62| 24.08| 67.66| 53.55| 52.96| 44.41| $51.39|47.85| 50.06|53.83| 38.83|46.65|=$ PED040] 20| 59.68| 47.31| 29.76| 58.55| 53.30| $50.50|53.92| 55.32|53.98| 54.07|45.66| 44.00|48.26|=$ PED040] 22| 56.88| $52.02|23.16| 71.19|55.18| 47.41|49.83| 49.63|49.36| 57.42|52.94| 47.05|46.84|=$ PED040] 23| 53.89| 48.16| $23.53|74.33| 50.64|53.73| 50.92|55.17| 50.05|56.90| 55.45|47.57| 49.36 \mid=$ PED040] 21|58.27|51.58| $22.01|60.72| 52.50|54.29| 49.97|53.66| 53.05|51.77| 48.46|47.10| 43.29 \mid=$ [ PED040] 23| 56.18| 55.04| $22.05|82.37| 62.43|56.72| 54.15|58.37| 54.73|60.39| 55.60|53.47| 54.34 \mid=$ [ PED040] 21|53.36|54.08| 23.46| 73.37| 55.20| 48.27| 49.94|55.68| 50.39|49.48| 53.87| 48.45| 48.07|= [ PED040] 22| 47.71| 49.94|22.85|67.11| 47.94|47.74|46.66| 50.29| 47.92| 50.70| 47.80|49.23|41.42|= [ PED040] 21|49.33|42.88| 21.59| 64.58| 44.82| 43.83| 42.93| 42.56| 44.01| 45.13| 45.57| 42.56| 38.51|= PED040] 22| $44.35|42.33| 19.60|67.21| 45.96|49.31| 45.12|50.81| 44.21|44.22| 43.97|44.95| 42.43 \mid=$ PED040] 22| 49.57| 55.66| $22.97|69.03| 52.75|51.01| 47.75|53.68| 47.64|50.29| 47.94|45.70| 48.30 \mid=$ [ PED040] 14|30.29| 28.95| 15.10| 45.83| 30.92| 32.09| $26.88|31.94| 29.47|31.68| 26.85|23.51| 30.79 \mid=$ PED029 [ P
PED030 [ P PED031 [ PED032 [ PED033 PED034 PED035 PED036 PED037 PED038 PED039 PED040 [ PED040] 23| 37.83| 32.90| 35.47| 50.23| 42.52| 40.84|39.63| 41.84| 42.05|37.37|32.54|35.33|31.92|DOR023 RON001 [ RON035] 20| 37.72| 36.53| 39.24| 13.72| 41.14| 39.45| 39.83| $56.41|37.99| 37.02|35.76| 41.30|47.16|=$

RON002 [ RONO35] 24|57.02| 55.13| 57.84| 22.07| 56.04| 56.27| 57.11| $71.66|51.91| 52.43|54.39| 53.87|51.83|=$ RONOO3 [ RONO35] 23| 43.07| 49.27| 54.94| 17.72| 46.53| 48.73| 50.06| 58.93| 47.97| 48.89| 47.26| 46.32| 47.08|= RONOO4 [ RONO35] 23| 45.38| 51.48| 58.13| 13.43| 50.63| 53.50| 55.99| 68.56| 46.23| 46.11| 52.71| 44.24| 47.46|= RONOO5 [ RONO35] 20| 43.19| 51.98| 45.76| 15.71| 34.97| 51.26| 45.61| 55.80| 45.08| 45.45 43.41| 45.54| 47.30|= RONO06 [ RONO35] 24| 45.10| 52.45 | $54.47|21.74| 46.26|53.30| 52.05|64.13| 51.58|46.57| 42.12|49.09| 48.51 \mid=$ RONOO7 [ RONO35] 21| 43.29| 47.20| 52.18| 16.24| 41.64| 51.44| 48.24| 56.89| 50.34| 45.03| 44.52| 43.12| 45.91|= RONO08 [ RONO35] 24| 51.70| 56.33| 53.48| 20.98| 48.87| 46.13| 47.80| 61.84| 46.05| 47.86| 46.95| 45.11| 45.37|= RONOO9 [ RONO35] 22| 45.32| 51.09| 45.84| 22.13| 45.03| 43.84| 50.93| 64.81| 46.59| 42.52| 41.18| 44.22| 45.93|= RON010 [ RONO35] 23| 48.21| 47.55| 50.41| 17.19| 48.30| 50.36 | 50.91 | 66.25 | $50.31|41.69| 42.97|47.50| 51.90 \mid=$ RON011 [ RONO35] 18| 37.24| 35.44| 38.71| 17.96| 33.35| 35.19| 37.71| 44.87| 39.65| 36.13| 37.52| 39.57| 36.44|= RON012 [ RONO35] 20| 43.03| 46.22| 48.15| 14.57| 39.63| 40.38| 38.82| 52.72| 44.14| 42.00| 39.62| 40.01| 47.47|= RON013 [ RONO35] 22| 43.79| 48.52| 52.40| 16.41| 47.46| 50.26| 48.68| 59.92| 45.98| 42.20| 45.56| 47.44| 52.14|= RONO14 [ RONO35] 20| 41.51| $51.17|45.35| 14.07|37.20| 47.89|49.40| 58.93|42.33| 43.45|38.42| 45.89|45.72|=$ RON015 [ RONO35] 21| 42.35| 49.05| 47.30| 14.37| 40.54| 46.86| 44.02| 59.06| 48.29| 39.74| 40.35| 42.45| 47.49|= RON016 [ RONO35] 23| 44.66| 52.46| 53.13| 14.18| 45.57| 50.57| 48.50| 62.70| 46.62| 45.18| 47.36| 45.17| 43.41|= RON017 [ RONO35] 20| 36.49| 38.73| 42.01| 12.61| 37.76| 39.50| 38.54| 46.76| 39.36| 37.43| 31.60| 40.17| 43.72|= RONO18 [ RONO35] 21| 39.55| 41.49| 47.99| 18.04| 43.07| 41.90| 42.91| $54.23|43.19| 37.35|35.49| 41.67|38.37|=$ RONO19 [ RONO35] 22| 42.83| 38.68| 45.67| 14.23| 43.85| 43.83| 46.29| 56.85| 44.42| 38.48| 32.84| 41.97 45.46|= RONO20 [ RONO35] 22| 46.36| 51.50| 49.69| 18.96| 42.08| 52.34| 49.24| 59.47| 44.64| 40.74| 48.81| 51.12| 47.74|= RONO21 [ RONO35] 22| 41.47| 47.28| 55.49| 14.92| 45.11| 44.33| 46.97| 60.75| 47.33| 39.81| 39.39| 41.34| 43.21|= RONO22 [ RONO35] 23| 49.15| 50.15| 53.49| 13.20| 49.54| 51.79| 51.01| 65.93| 50.84| 51.06| 51.73| 47.44| 47.84|= RONO23 [ RONO35] 24| 50.81| 55.43| 62.01| 22.05| 56.01| 53.37| 52.87| 69.18| 56.28| 48.02| 44.43| 47.37| 52.84|= RONO24 [ RONO35] 23| 52.07| 50.43| 52.82| 18.63| 56.18| 47.02| 48.34| 66.97| 49.38| 41.52| 44.25| 49.25| 51.96|= RONO25 [ RONO35] 23| 43.86| 43.92| 52.20| 19.45| 44.50| 46.07| 45.66| 62.15| 45.27| 43.07| 40.25| 41.74| 48.36|= RONO26 [ RONO35] 22| 44.55| 45.05| 53.15| 17.57| 43.47| 44.94| 43.73| 58.93| 42.27| 44.84| 52.42| 41.04| 43.83|= RONO27 [ RONO35] 21| 39.12| 42.62| 48.39| 15.90| 41.92| 43.21| 42.37| 54.97| 41.96| 41.70| 38.28| 40.61| 43.16|= RONO28 [ RONO35] 22| 46.25|51.61| 52.12| 18.52| 48.57| 48.70| 48.73| 68.30| 49.02| 44.45| 41.57| 43.98| 46.26|= RONO29 [ RONO35] 22| 44.00| 46.76| 52.49| 19.44| 52.37| $51.39|48.20| 59.23|53.84| 43.16|41.35| 47.84|49.08|=$ RONO30 [ RONO35] 23| 44.32| 41.41| 50.11| 18.44| 53.02| 50.48| $51.27|66.77| 51.72|42.87| 43.21|46.10| 47.53 \mid=$ RONO31 [ RONO35] 23| 48.36| 49.53| 53.93| 19.13| 54.67| 52.51| 50.88| 65.61| 51.94| 43.70| 50.14| 45.88| 51.05|= RON032 [ RONO35] 20| $35.53|39.34| 43.55|16.62| 39.20|46.06| 46.26|55.81| 45.73|37.11| 33.10|40.42| 44.98 \mid=$ RON033 [ RONO35] 22| 53.38|53.36|57.73| 24.95| 55.96| 56.86| $54.58|67.73| 54.92|50.80| 51.94|47.28| 53.28 \mid=$ RONO34 [ RONO35] 19| 37.19| 42.36| 43.03| 18.45| 34.22| 39.87| 42.83| 47.10| 40.73| 40.11| 37.10| 41.60| 39.70|= RONO35 [ RONO35] 23| 45.41| 49.74| 53.30| 15.26| 52.60| 50.47| 53.47| 69.29| 47.18| 44.14| 48.50| 47.65| 51.51|= VANOO1[ VANO32] 13| 29.06| $31.02|33.58| 26.43|21.48| 29.40|30.07| 30.25|30.17| 33.44|31.48| 29.01|29.77|=$ VANOO2[ VANO32] 14| 29.94| 27.79| 24.10| 29.11| 13.38| 19.57| 18.47| 29.14| 16.92| 25.68| 22.27| 23.77| 22.88|= VANOO3[ VANO32] 12| 20.74|27.28| 26.65| 32.53| 12.14| 22.62| 28.23| $31.77|26.55| 31.06|28.58| 29.40|25.43|=$ VAN004[ VAN032] 12| $31.46|31.24| 30.37|29.15| 23.37|37.72| 32.44|39.73| 29.76|34.35| 32.53|26.68| 24.57 \mid=$ VANOO5[ VANO32] 10| 15.85|22.65|21.19| 18.08| 8.46| 16.66| 16.27| 19.12| $16.03|17.28| 20.54|12.94| 16.12 \mid=$ VANOO6[ VANO32] 14|31.33|31.58| 28.41|37.90| 12.88| $30.22|25.94| 36.37|20.70| 29.74|32.79| 22.93|24.69|=$ VANOO7[ VANO32] 11| $22.10|25.21| 21.71|25.77| 10.50|18.57| 11.63|19.47| 12.51|19.60| 19.19|17.73| 19.44 \mid=$ VANOO8 [ VANO32] 15| 19.90| 28.17| 27.32| $32.80|12.12| 24.84|20.03| 22.70|23.89| 22.58|28.56| 25.56|19.80|=$ VANOO9 [ VANO32] 21| 29.89| 38.86|37.77| 46.76| 18.76| 28.12| 29.56| $30.79|33.32| 39.87|35.10| 30.58|34.16|=$ VANO10 [ VANO32] 20| $25.12|33.61| 36.72|32.44| 38.60|30.70| 35.31|35.26| 39.99|39.00| 35.75|36.12| 33.01 \mid=$ VANO11 [ VANO32] 21|35.08|39.04|39.92| 49.96| 15.80| $38.81|36.21| 44.32|43.21| 45.63|43.84| 34.05|36.67|=$ VANO12 [ VANO32] 11| 18.91| $22.71|27.72| 32.02|9.42| 19.67|20.28| 22.24|18.37| 23.87|25.43| 21.81|18.47|=$ VANO13[ VANO32] 17| 35.17| 37.08| 38.19| $44.85|24.25| 33.80|31.42| 42.46|34.74| 33.76|39.55| 29.59|30.51|=$ VANO14 [ VANO32] 10| 19.82| 27.03| 23.78| 27.05| 10.35| 22.11| 18.20| 23.39| 16.20| $21.24|24.96| 22.92|15.71|=$ VANO15 [ VANO32] 21| 28.15| $32.96|35.80| 42.61|18.00| 29.47|32.92| 31.52|36.88| 35.56|32.66| 32.67|32.02|=$ VAN016[ VANO32] 14| $28.80|34.83| 30.80|46.06| 23.82|26.32| 27.94|30.29| 28.43|32.38| 37.59|21.52| 27.70 \mid N A M 034$ VANO17[ VANO32] 14|21.95|20.50| 19.02| $31.29|13.77| 17.71|18.32| 25.84|15.78| 26.04|24.09| 23.13|21.76|=$ VANO18[ VANO32] 14| $26.23|37.98| 34.25|36.50| 19.45|34.92| 25.67|29.03| 24.98|30.63| 29.64|30.86| 22.61 \mid=$ VANO19 [ VANO32] 16| $28.06|29.26| 27.36|27.63| 13.09|22.22| 22.45|28.70| 22.34|27.88| 24.57|24.48| 22.01 \mid=$ VANO2O[ VANO32] 15| $27.96|33.86| 36.84|36.24| 15.33|22.93| 25.38|36.44| 29.41|34.44| 34.79|23.17| 31.18 \mid=$

VANO21[ VANO32] 19| 42.01|39.61|36.91| 42.65| 15.94| 35.66| 29.81| $41.61|31.90| 40.94|40.68| 30.75|33.56|=$ VANO22 [ VANO32] 20|34.87|38.24|41.98|51.44| 17.37|37.66|36.27|46.83|41.02| 43.20|38.74|35.94|37.63|= VANO23 [ VANO32] 12| $24.77|27.10| 30.04|35.20| 13.08|27.35| 21.55|31.65| 27.16|28.38| 32.70|26.80| 28.14 \mid=$ VANO24 [ VANO32] 12| 21.07| 23.92| 26.24| 26.74| 11.22| 23.44| 16.47| 27.40| 20.69| 22.47| 25.89| 19.07| 20.12|= VANO25[ VAND32] 14|18.65|24.62|21.58| 29.05| 9.22| 9.61|14.57|22.12| 11.46| 17.94| 17.67| 20.52| 17.37|= VANO26[ VANO32] 14|22.07| 23.84| $27.98|28.39| 11.97|24.23| 23.40|30.90| 26.13|28.92| 29.92|20.05| 21.55 \mid=$ VANO27[ VAN032] 19|35.84|43.29| 42.14| $53.15|24.02| 33.31|34.51| 46.63|35.86| 42.66|41.11| 35.24|31.71|=$ VANO28 [ VANO32] 15|31.98|30.57| 28.20| $36.58|11.25| 27.02|21.30| 35.05|19.36| 25.72|27.81| 25.58|23.59|=$ VAN029 [ VANO32] 22| $31.78|37.23| 34.67|50.83| 19.32|35.37| 32.61|40.15| 32.67|39.08| 35.76|33.96| 32.25 \mid=$ VANO32] 19| 30.43 | $35.98|34.20| 39.70|14.88| 30.18|27.94| 31.32|32.43| 30.87|32.08| 29.60|29.88|=$ VANO32] 20| 33.44| $30.25|31.27| 48.25|19.22| 29.66|28.95| 33.08|27.85| 32.20|35.65| 35.15|27.70|=$ VANO32] 21| 37.09| 43.18| 35.23| 42.62| 21.92| 28.97| 26.98| 31.15| 32.18| 31.83| 30.22| 28.10| 23.78|= SWP001[ SWP019] 19| 29.17|35.68| 29.32| 40.05| 23.50| 17.31|21.60|27.06|21.47|22.01|29.46|29.28|22.61|= SWP002[ SWP019] 18| 26.19| $31.20|30.82| 39.19|23.87| 13.64|21.60| 28.42|19.38| 26.27|28.54| 26.21|23.54|=$ SWP003[ SWP019] 19| 40.29| 46.65| 44.74| 48.14| 34.74| 23.99| 38.58| 47.91| 30.94| 42.93| 44.80| 32.43|34.19|= SWP004 [ SWP019] 18| 37.48| 44.81| 40.45|56.99| 35.18| 26.62| 38.50| 34.84| 32.23| $41.50|38.20| 36.75|38.34|=$ SWP005 [ SWP019] 21| $35.48|42.03| 41.30|48.84| 43.28|26.19| 32.93|46.94| 28.60|42.13| 42.44|35.59| 32.35 \mid=$ SWP006[ SWP019] 16| 28.61| 35.55| 30.73| 42.06| 23.60| $17.34|23.13| 24.30|30.15| 30.67|28.82| 30.24|20.78|=$ SWP007[ SWP019] 18| 28.25|37.51| $35.00|40.72| 35.22|20.68| 22.37|34.05| 25.47|28.29| 36.51|32.14| 29.16 \mid=$ SWP008[ SWP019] 19| 37.00| $45.29|40.82| 51.61|37.60| 32.73|32.30| 33.37|36.20| 41.08|37.07| 37.50|40.46| S S P 033$ SWPO09 [ SWP019] 17| $23.82|32.74| 28.14|38.88| 25.97|18.23| 22.88|28.00| 17.35|21.15| 24.34|23.44| 23.43 \mid R S P 026$ SWP010 [ SWP019] 19| 32.03| 42.92| 45.16| 47.05| 36.60| 17.93| 30.81| 34.29| 28.89| 33.68| 40.91| 37.31| 26.38|= SWP011[ SWP019] 18| $21.90|30.90| 27.85|34.87| 16.93|15.73| 25.55|25.92| 23.22|23.00| 24.11|27.01| 22.86 \mid=$ SWP012[ SWP019] 18| 37.28| 47.39| 46.48| 51.17| 36.27| 17.20| 26.88| $41.40|26.50| 40.79|40.31| 37.85|36.06|=$ SWP013[ SWP019] 18| 39.96| 40.96| 44.65| 45.87| 34.38| $22.42|34.65| 39.89|34.57| 35.62|39.44| 35.39|34.16|=$ SWP014 [ SWP015 [ SWP016 [ SWP017 [ SWP018 [ SWP019 [ SSP001[ SSP002[ SSP033] 16| $34.15|35.32| 31.31|44.92| 29.83|25.53| 28.44|29.64| 25.42|31.57| 36.25|25.29| 34.44 \mid N A M 034$ SSPO03[ SSPO33] 14| $30.18|37.05| 29.18|29.09| 24.65|17.35| 20.97|31.58| 22.23|26.65| 28.97|23.60| 32.02 \mid S W P 019$ SSP004[ SSP033] 16| $34.30|38.31| 36.61|49.51| 29.42|26.40| 32.09|28.97| 26.96|34.33| 35.56|28.38| 32.16 \mid S W P 019$ SSP005[ SSP033] 16| 25.79| 33.63| 28.89| 38.18| 20.86| 28.38| 14.96| 21.92| 21.70| 25.65| 26.40| 25.65|34.42|= SSP006[ SSP033] 16|21.13|34.60|24.82| $38.52|17.84| 18.81|8.21| 19.10|10.35| 20.22|20.89| 24.32|24.11|=$ SSP007[ SSP033] 16|22.98| $32.31|23.52| 38.60|17.18| 15.05|13.56| 16.14|15.05| 23.50|23.84| 23.18|20.19|=$ SSP008[ SSP033] 15| 19.84| 33.47| 23.36| 42.06| 19.08| 17.76| 11.27| 15.07| 11.99| 22.69|21.84|26.35|22.85|= SSP009[ SSP033] 16| 25.96| $30.27|26.74| 40.70|19.45| 23.24|10.63| 24.18|16.91| 23.68|26.66| 24.60|24.04|=$ SSP010 [ SSP033] 16| 23.85|34.05|26.43|42.41|24.09| 12.77|11.30| 15.02| 16.25|22.64|24.65|19.91|23.33|= SSP011[ SSP033] 15| 14.69|28.96|21.68| $30.38|22.21| 14.86|11.68| 13.15|18.10| 20.04|20.17| 26.32|19.04|=$ SSP012[ SSP033] 15| 22.75|34.49| 22.60| $37.63|21.43| 18.72|11.46| 19.86|18.09| 20.40|26.26| 23.73|26.25|=$ SSP013[ SSP033] 15|21.64|28.64|24.05|36.40|21.29| 22.14| 9.48| 18.36| 15.65| 18.56| 19.79|23.64|25.11|= SSP014[ SSP033] 16|26.73|32.12|24.71|39.58| 18.14|20.35|11.04|23.39|13.73|22.30|25.43|23.33|24.62|= SSP015[ SSP033] 15| 18.28| $28.17|19.53| 31.93|19.37| 18.69|13.23| 19.17|11.64| 15.45|17.19| 18.23|18.95| R S P 026$ SSP016[ SSP033] 16|22.56|31.34|25.04|42.50|24.34|22.46| 12.95|21.45|15.82|20.99|20.89|28.78|25.85|= SSP017[ SSP033] 16| 38.81| $44.25|40.42| 50.32|36.37| 29.45|31.17| 36.90|23.91| 40.07|33.72| 33.27|34.87| R S P 026$ SSP018[ SSP033] 16|22.21|35.93|28.14|35.88|17.65|23.93|14.68|17.58|18.15|22.09|20.66|25.48|22.49|= SSP019[ SSP033] 16| 24.03| $36.89|24.32| 39.90|16.25| 20.55|12.01| 25.45|15.43| 23.03|23.81| 25.54|24.13|=$ SSP020 [ SSP033] 13| 14.72| 21.52 | 18.54 | $23.02|12.41| 18.42|14.34| 11.25|16.86| 14.75|14.66| 17.71|15.03|$ RKPO28 SSP021[ SSP033] 14| 19.01|31.89| 24.46| 36.70| 19.69| 17.26| 12.57| 18.18| 15.01| 20.90| 21.19| 19.35|24.04|= SSPO22 [ SSP033] 15|14.95|30.22| 24.07| 32.67| 21.61| 16.11| $8.42|14.06| 13.62|20.13| 21.45|21.00| 22.38 \mid=$ SSP023[ SSP033] 16|23.96| 34.41|26.22| $35.78|15.96| 23.85|8.81| 17.87|12.94| 22.99|22.77| 24.74|25.24|=$

SSP024 [ SSP033] 14| 16.01|30.09| $20.67|34.11| 18.35|11.52| 8.94|13.84| 12.62|19.61| 20.75|18.03| 14.56 \mid=$ SSP025 [ SSP033] 15| $28.34|32.94| 27.03|38.92| 22.40|22.98| 12.49|20.49| 21.07|23.97| 27.73|22.40| 29.43 \mid=$ SSP026 [ SSP033] 15| 31.17|38.63|35.18|36.82| $27.39|19.08| 24.69|31.25| 24.54|28.40| 33.54|26.10| 32.84 \mid S W P 019$ SSP027 SSP028 [ SSP033] $15|29.76| 42.45|36.63| 38.30|25.43| 27.58|25.11| 31.74|24.80| 29.31|30.59| 26.61|30.75| R S P 026$

SSP029 SSP030 [ SSP031 [ SSP032 [ SSP033 [ RKP001 [ RKP002 [ RKP003 [ RKP004 [ RKP005 [ RKP006 [ RKP007 [ RKP008 RKP009 [ RKP010 [ RKP011 [ RKP012 [ RKP013 [ RKP014 [ RKP015 [ RKP016 RKP017 [ RKP018 [ RKP019 [ RKP020 [ RKP021 [ RKP022 [ RKP023 [ RKP024 [ RKP025 [ RKP026 [ RKP028 RSP001 [ RSP002 [ RSP003 [ RSP004 [ RSP005 [ RSP006 RSP007[ RSP008 [ RSP009 [ RSP010 [ RSP011 [ RSP012 [ RSP013 RSP014 [ RSP015 [ RSP016 [ RSP017 [ SSP033] $16|18.78| 33.13|21.64| 35.91|19.86| 16.98|12.49| 17.22|14.37| 20.57|25.20| 21.65|16.57|=$
SSP033] $14|15.59| 28.55|24.42| 31.69|18.75| 20.27|10.17| 14.88|16.36| 18.63|17.45| 23.34|23.84|=$ SSP033] $14|34.95| 38.26|36.98| 37.19|27.46| 30.12|24.78| 30.60|24.33| 33.98|33.78| 23.01|33.91|$ |NAM034 SSP033] $14|18.94| 26.90|23.93| 36.48|23.77| 18.58|13.44| 15.43|19.98| 20.25|19.82| 27.44|23.59|=$ SSP033] 16| $26.30|36.22| 28.30|47.05| 26.97|15.52| 12.60|16.91| 19.25|23.52| 26.63|23.35| 25.48 \mid=$ RKP028] 20| $29.19|38.07| 36.63|45.82| 22.42|26.25| 24.93|18.12| 29.79|31.40| 29.75|28.81| 23.19 \mid=$ RKP028] 19| 49.14| 48.97| 45.92| 51.79| 40.16| 40.97| 32.68| $30.29|39.94| 45.24|44.33| 43.25|46.57|=$ RKP028] 20| $27.63|40.66| 30.34|46.84| 27.03|24.84| 21.82|13.22| 27.40|30.42| 29.25|28.49| 29.78 \mid=$ RKP028] 20| $31.55|33.07| 32.03|55.36| 32.26|30.56| 29.95|20.42| 38.31|37.67| 30.69|31.98| 28.57 \mid=$ RKP028] 18| $26.69|31.65| 27.51|39.55| 25.15|23.16| 20.63|16.53| 28.55|29.25| 30.48|27.94| 23.16 \mid=$ RKP028] 18| 30.17| 33.56| 29.53| 44.94| 23.32| $24.73|25.18| 14.93|31.76| 39.20|36.86| 33.98|30.82|=$ RKP028] 20|31.21| 42.24|35.39| 54.93| 31.63| $21.44|22.30| 13.21|28.66| 37.10|32.51| 29.70|32.25|=$ RKP028] 19| 30.02| 41.15| 30.11| $51.06|28.09| 29.23|20.93| 12.12|27.24| 31.76|30.15| 28.60|30.68|=$ RKP028] 17| 30.70| $40.72|39.28| 52.61|32.26| 29.35|30.64| 20.21|32.82| 41.68|38.39| 32.32|37.24|=$ RKP028] 19| 30.20|38.32| 33.98| $56.91|36.21| 31.94|29.06| 16.00|33.44| 35.67|34.50| 33.80|41.53|=$ RKP028] 18| $25.95|32.32| 31.39|54.47| 30.93|25.44| 29.87|17.14| 24.77|35.65| 35.51|29.83| 29.99 \mid=$ RKP028] 19| $31.49|35.97| 26.50|50.68| 26.57|24.41| 21.30|11.03| 25.42|27.69| 25.63|26.80| 25.96 \mid=$ RKP028] 20| 42.19| $54.17|39.69| 55.22|36.48| 35.71|31.53| 25.24|35.82| 42.24|41.93| 35.79|39.27|=$ RKP028] 17| 32.39| 30.46| 26.74| 43.86| 24.10| $21.78|21.40| 13.52|23.37| 25.53|29.40| 29.60|22.61|=$ RKP028] 18| $30.45|33.69| 34.84|46.86| 29.71|31.82| 27.06|16.39| 36.23|31.93| 28.42|32.33| 34.02 \mid=$ RKP028] 22| 47.28| 52.43| 42.80| 70.33| 47.61| 40.92| 29.38| $22.78|37.56| 41.75|42.31| 34.95|47.51|=$ RKPO28] 19| $44.32|47.43| 39.88|58.62| 41.28|33.80| 31.06|22.41| 38.54|41.35| 42.12|41.16| 42.30 \mid=$ RKP028] 16| 17.87| 27.91| $21.29|35.24| 18.81|14.85| 14.60|10.30| 16.05|21.51| 18.54|19.77| 19.84 \mid=$ RKP028] 19| 33.81| 44.72 | $34.78|57.57| 33.84|24.96| 19.85|15.13| 26.98|33.77| 37.48|31.81| 33.02 \mid=$ RKP028] 19| $40.28|36.67| 37.92|58.86| 36.14|32.08| 27.92|21.64| 30.23|37.22| 41.41|29.98| 34.05 \mid=$ RKP028] 19| $27.72|32.87| 31.19|43.52| 22.21|21.13| 19.86|16.67| 27.39|29.33| 29.19|31.44| 24.41 \mid=$ RKP028] 20| $23.90|37.19| 32.01|47.79| 25.97|26.30| 25.88|17.80| 32.32|31.63| 25.98|33.46| 26.28 \mid=$ RKP028] 20| $33.53|42.45| 40.31|52.76| 34.11|32.41| 21.17|16.96| 36.16|31.72| 33.18|32.36| 32.39 \mid=$ RKP028] 21| $31.71|46.89| 37.59|59.63| 30.70|32.40| 27.02|17.15| 31.90|36.36| 35.96|37.09| 34.66 \mid=$ RKP028] 19| 29.55| 31.65|26.88| 51.16| 23.84| $25.55|24.98| 15.84|33.89| 36.32|37.33| 34.34|29.51|=$ RKP028] 18| $21.91|31.79| 28.67|46.43| 24.78|23.36| 19.08|11.73| 23.32|32.99| 33.40|24.58| 25.98 \mid=$ RKP028] 17| 33.50| 45.81| 39.34| $44.89|27.08| 22.88|23.03| 25.31|27.33| 34.93|33.30| 26.91|34.83| S W P 019$ RSP026] $16|30.90| 33.95|25.83| 38.55|21.02| 20.13|20.30| 27.35|15.11| 29.57|26.75| 23.49|20.90|=$ RSP026] 13| $23.37|30.51| 19.93|28.52| 17.35|15.09| 14.29|17.41| 9.14|23.45| 20.70|18.60| 16.64 \mid=$ RSP026] $8|15.47| 18.07|11.72| 20.14|9.49| 9.58|8.41| 6.82|4.54| 14.36|12.51| 14.02|9.83|=$ RSP026] 15| $22.11|32.22| 26.74|32.61| 15.32|18.82| 14.61|23.55| 7.99|15.15| 15.81|22.22| 18.97 \mid=$ RSP026] 15| $21.65|28.79| 22.34|28.07| 18.13|18.15| 11.46|22.90| 7.52|19.30| 18.34|18.52| 18.22 \mid=$ RSP026] 8| 10.49| 12.40| $12.74|12.18| 8.48|8.61| 6.40|7.79| 5.61|10.58| 9.52|11.70| 10.43 \mid=$ RSP026] 12| 19.73 | $27.14|21.27| 25.92|16.82| 13.39|10.28| 15.31|8.01| 15.18|15.76| 16.44|16.48|=$ RSP026] 14| 19.03| $27.23|23.06| 30.73|17.29| 16.76|15.74| 20.54|11.88| 20.24|18.33| 23.36|17.77|=$ RSP026] 16| $23.59|32.97| 26.55|32.82| 18.41|21.00| 11.45|23.51| 10.48|21.60| 22.42|22.76| 18.90 \mid=$ RSP026] $13|18.87| 29.14|22.02| 29.57|14.54| 11.26|7.35| 12.56|7.77| 17.91|19.51| 16.88|16.67| S S P 033$ RSP026] $13|17.96| 23.13|19.00| 29.46|12.27| 13.61|6.90| 14.98|7.74| 14.05|16.26| 14.55|14.48| S S P 033$ RSP026] 13| 18.99 | $28.33|21.88| 28.53|15.64| 15.90|9.00| 17.55|7.26| 16.51|15.75| 17.72|17.26|=$ RSP026] 16| $26.69|37.09| 23.35|35.83| 19.07|20.70| 16.07|25.97| 9.34|25.04| 23.84|18.45| 19.50 \mid=$ RSP026] $13|21.65| 25.98|23.24| 32.70|17.33| 17.11|9.12| 20.32|7.39| 18.89|19.64| 22.15|18.91|=$ RSP026] 14|21.12|35.28|21.51|33.94| 16.93| 15.18|14.24|22.77| $8.66|22.15| 17.74|20.81| 16.81 \mid=$ RSP026] $9|17.11| 20.40|19.78| 20.58|13.82| 10.92|9.58| 17.02|6.06| 13.76|12.66| 13.58|14.61|=$ RSP026] $13|18.07| 25.83|22.56| 28.42|13.21| 18.86|16.79| 24.87|14.41| 17.51|21.05| 16.62|16.48| V A N 032$

RSP018[ RSP026] 9| 15.03| 16.48| 19.82| 17.22| 15.76| 13.86| 16.45| 15.01| $15.42|14.73| 17.14|12.10| 17.60 \mid$ NAM034 RSP019 [ RSP026] 14|25.19|25.81| $21.44|28.52| 16.36|17.70| 15.45|26.01| 10.13|19.36| 18.11|13.28| 18.32 \mid=$ RSP020 [ RSP026] 13| 20.34| 29.09| 17.16| 27.88| 14.68| $11.97|13.22| 16.43|11.64| 21.30|19.55| 18.22|17.27|=$ RSP021 [ RSP026] 12| 25.23| 35.02| 21.96| 32.52| 24.45| 16.06| 18.30| 20.76| 15.81| 26.12| $25.86|18.72| 20.81 \mid=$ RSP022 [ RSP026] 14| 29.00| 32.75| 30.76|35.66| 29.38| $24.20|27.79| 29.06|22.86| 29.94|33.57| 20.92|24.98|$ NAM034 RSP023 [ RSP026] 12| $23.38|27.83| 20.67|27.95| 18.60|17.68| 12.02|17.57| 9.35|14.86| 19.70|17.38| 19.62 \mid=$ RSP024 [ RSP026] 11| 18.70| $22.85|17.58| 15.86|9.81| 12.25|9.41| 10.28|6.10| 14.18|12.93| 15.24|11.41|=$ RSP025 [ RSP026] 12| 28.99| 32.02| 26.14| 33.54| $23.58|22.87| 19.17|25.02| 12.64|18.81| 21.49|18.04| 23.67 \mid=$ RSP026 [ RSP026] 14| 23.70| $30.86|23.78| 25.43|15.89| 19.73|17.87| 24.58|9.60| 20.57|16.03| 17.22|16.37|=$ ZUL001 [ ZUL035] 12| 15.38| 19.15| 21.77| 30.55| 20.54| $23.80|21.00| 22.30|18.73| 11.06|15.10| 17.17|21.02|=$ ZUL002 [ ZUL035] 14| 23.49| 26.22| 23.77| 38.69| 26.25| $27.95|30.50| 25.52|30.31| 14.12|19.42| 23.41|24.98|=$ ZUL003 [ ZULO35] 19| 37.17| 37.15| 32.56| 43.53| 39.57| 39.00| 37.39| 41.39| 42.09| 25.46| 31.07| 35.61| 41.42|= ZUL004 [ ZUL035] 21| 29.67| 43.36| 39.49| 47.96| 46.02| $38.45|35.72| 47.37|38.84| 17.83|27.53| 35.35|31.97|=$ ZUL005 [ ZUL035] 16| 31.99|30.28| 34.65| 35.44| 37.92| 35.55| $36.64|42.03| 38.16|17.81| 24.43|31.56| 34.12 \mid=$ ZUL006 [ ZUL035] 14| 28.23| 30.44|31.54|34.59| 30.56| $28.00|24.80| 32.64|23.02| 16.46|24.75| 24.51|23.79|=$ ZUL007 [ ZUL035] 17| 33.58|41.92| 32.93|39.77|31.46|33.71|31.92|32.56|32.97|20.64|30.01|30.95|28.67|= ZUL008 [ ZUL035] 19| 28.70| 37.25| 27.89| 43.66| 34.08| 33.76| 28.43| 33.91|31.36| 15.12| 21.71|28.34|26.86|= ZULO09 [ ZUL035] 20|27.05|39.15| 29.33| 40.87| 34.26| 38.46| 35.33| 35.75|34.13|17.99|21.85|34.08| 26.55|= ZUL010 [ ZUL035] 21| 43.82| 42.12| 38.25| 46.67| 46.11| 43.77| 43.41| 51.10| 40.94|25.19| 33.16|40.30|40.49|= ZUL011 [ ZUL035] 14|21.32| 24.90| 27.33| 29.86| 23.59| 20.60| $22.94|26.34| 23.72|12.79| 17.08|20.80| 22.15 \mid=$ ZUL012 [ ZUL035] 16| 29.82| 28.66| 32.00| 46.37| 35.44| 33.76| 32.84|36.19|31.82| $22.85|26.46| 33.04|28.70|=$ ZUL013 [ ZUL035] 12| 20.10| 14.58| 19.14| 29.77| 21.67 | $22.68|22.02| 23.57|24.41| 12.87|19.98| 17.69|19.46|=$ ZUL014 [ ZUL035] 21|27.51|39.72| 32.12| 43.57| 36.74| 32.61| 29.36| 41.75|35.42| 20.49| 29.08|33.72| 37.67|= ZUL015 [ ZUL035] 18| 27.00| 40.17| 37.48| 47.72| 37.36| 39.54| 36.74| 42.63| 36.20| 18.53| 25.17| 30.56| 34.55|= ZUL016 [ ZUL035] 19| 35.81| 43.92| 41.13| 46.11| 40.22| 41.58| 37.72| 45.40| 41.16| 19.04|33.89|33.78| 41.95|= ZUL017 [ ZUL035] 14| 22.98|31.72|34.46| 41.74|28.44|26.66|23.64|26.61|25.11|16.77|25.77|25.55|23.78|= ZUL018 [ ZUL035] 17| 38.83| 40.17| 42.65| 52.62| 39.98| 33.70| $36.13|45.89| 36.33|23.30| 38.01|32.63| 35.86 \mid=$ ZUL019 [ ZUL035] 14|26.40| 23.65| 27.89| 39.91| $24.24|27.81| 28.85|27.44| 30.20|15.83| 24.98|23.82| 24.60 \mid=$ ZUL020 [ ZUL035] 19| 36.65|38.33| 31.43| 50.63| 34.79| 35.71| 33.57| 35.32| 31.49| 20.02| 30.43|32.77| 24.02|= ZUL021 [ ZUL035] 19| 29.32| 36.40| 30.69| 41.95| 34.85| 39.86| 38.86| 44.23| 41.04| 15.50| 25.05|36.02| 30.60|= ZUL022 [ ZUL035] 20| 29.02| 40.66| 34.56| 36.68| 38.69| 34.62| 35.11| 44.19|34.50| 18.36| $24.48|36.24| 29.23 \mid=$ ZUL023 [ ZUL035] 19| 29.29|33.89| 35.94|33.20|36.75| 36.89| 36.97| 43.49| 40.42| 18.74|22.78|32.34|34.21|= ZUL024 [ ZUL035] 20| $36.88|39.53| 29.44|48.57| 39.59|36.47| 37.41|34.04| 40.13|24.75| 23.85|33.05| 30.10 \mid S W A 027$ ZUL025 [ ZUL035] 19| 30.34|35.79| 28.55| 36.59| $28.14|29.76| 23.87|36.24| 26.19|21.10| 24.88|26.62| 27.42 \mid=$ ZUL026 [ ZUL035] 19| 38.42| 35.43| 39.83| 50.36| 46.96| 37.50| 32.67| 48.02| 37.34| 23.50| 24.36|31.23| 40.88|= ZUL027 [ ZUL035] 20| 30.45|40.12| 28.88| 45.82| 29.40| 30.82| 31.13| 27.19| 37.19| 16.08| 24.08| 30.72| 26.30|= ZUL028 [ ZUL035] 20| 33.31| 39.34| 33.66| 43.88| 41.59| 37.89| 39.16| 47.09| 42.27|21.61| 29.95|35.65|38.48|= ZUL029 [ ZUL035] 20|31.90| 50.26| 34.95| 45.81| 41.38| 39.29| 36.24| 42.55|37.99| 19.44| 28.11|37.55|33.37|= ZUL030 [ ZUL035] 22| 38.68| 42.05| 31.48| 47.27| 39.02| 44.67|35.03| 48.09| 37.84|24.91|29.01|35.61|33.10|= ZUL031 [ ZUL035] 14| 19.80| 25.05| 26.02| 31.71| $26.15|22.39| 22.68|29.03| 17.52|15.26| 20.72|24.00| 18.36 \mid=$ ZUL032 [ ZUL035] 19| 36.12| 40.87| 34.44| 58.02| 38.48| 38.82| 33.10| $33.47|37.03| 18.79|34.68| 30.72|31.14|=$ ZUL033 [ ZUL035] 21| 32.05|38.76| 28.63| 46.24| 39.81| 38.33| 35.86| 47.23| 34.96| 17.37| 31.83| 40.73| 31.73|= ZUL034 [ ZUL035] 21|33.48|35.99| 35.20| 43.33| 40.26| 36.74|41.64|51.14|39.95|19.03|29.42|34.06|31.11|= ZUL035 [ ZUL035] 17| 24.21|35.80| 29.31| 40.49| 27.59| 25.18| $25.08|29.08| 27.80|15.45| 24.08|26.61| 28.40 \mid=$ SWA001 [ SWA027] 15| 19.22| 31.43| 30.27| $28.80|26.99| 29.07|29.29| 31.49|27.84| 19.13|14.46| 26.86|27.22|=$ SWA002 [ SWA027] 23| 38.52| 55.79| 47.59| 59.71| 44.37| 43.83| 35.95| 42.05|39.68|31.52|31.14|38.39| 41.75|= SWA003 [ SWA027] 24|44.78| 47.46| 47.88| 62.66| 55.59| 44.24| 45.59| 55.26| 50.05| 45.14|40.96| 48.49| 45.78|= SWA004 [ SWA027] 23| 52.76| 54.59| 61.29| 65.58| 64.95| $57.21|50.90| 61.43|53.25| 48.16|35.63| 44.65|61.76|=$ SWA005 [ SWA027] 24|54.74|55.74|57.63|76.95| 59.18| 59.09| 59.12| 68.27| 53.98| 52.40| 44.76| 49.09| 54.18|= SWA006 [ SWA027] 22| 39.70| 38.29| 36.36| 54.71| 51.02| 43.80| 46.18| 56.42| 46.32| 34.93| 22.68| 42.90| 35.96|= SWA007 [ SWA027] 22| 34.29| 40.62| 39.85| 46.63| 43.54| 40.18| 40.70| 49.77| 43.99|31.72| 23.19| 44.57| 36.57|= SWA008 [ SWA027] 24|45.93| 47.37| $51.25|57.03| 58.61|50.68| 49.65|61.45| 54.23|41.97| 30.50|48.64| 50.62 \mid=$ SWA009 [ SWA027] 22| 36.43| 39.43| 35.17| 44.36| 39.08| 40.27| 37.42| 43.20| 38.51| $28.99|19.85| 38.87|37.22|=$ SWA010 [ SWA027] 22| 40.45| 52.66| 40.70| 53.70| 47.15| 49.73| 42.69| 53.52| 50.17| 30.84| 26.70| 45.49| 44.96|=

SWA011[ SWA027] 24|39.81| 42.01| 45.70| $53.45|45.23| 45.56|42.52| 50.53|43.99| 38.26|27.74| 39.50|40.48|=$ SWA012 [ SWA027] 23| 28.85| 46.58| 42.03| 56.54| 40.76| 45.36| 42.16| $51.87|44.18| 36.81|24.96| 46.99|44.04|=$ SWA013 [ SWA027] 23| 49.52| 46.36| 44.90| 64.54| 54.81| 50.17| $50.44|61.36| 47.97|40.82| 33.41|46.94| 48.36 \mid=$ SWA014 [ SWA027] 22| 34.54| 40.78| 36.37| 43.76| 44.08| 37.44| 30.31| 45.52| 39.98| 23.89| 22.91| 39.76| 37.15|= SWA015 [ SWA027] 22| 36.37| 42.43| 32.62| 51.01| 37.52| 42.67| 45.15| 48.83| 44.01| 33.18| 24.07| 38.43| 34.54|= SWA016[ SWA027] 21| 29.98| 35.83| 34.29| 44.03| 38.05| 36.97| 41.02| 40.93| 37.09| 28.72| 17.47| 36.76| 34.26|= SWA017 [ SWA027] 22| 32.16| 48.82| 41.66| 50.66| 47.18| 38.13| 38.58| 49.38| 42.03| 33.18| $24.26|39.97| 38.09 \mid=$ SWA018 [ SWA027] 23| $38.38|38.06| 42.92|47.56| 46.52|41.07| 44.70|51.77| 46.72|39.50| 23.14|45.93| 42.48 \mid=$ SWA019 [ SWA027] 23| 37.04| 47.89| 39.28| 47.66| 44.88| 49.86| 47.41| 52.47| $51.69|28.96| 21.20|46.18| 42.86 \mid=$ SWA020 [ SWA027] 21|34.92| 41.56| 40.20| 47.55|37.11| 37.68| 36.42| 37.06| 39.41|32.09| 23.40|35.98| 36.72|= SWA021[ SWA027] 20| 41.31|45.02| 47.86| 55.83| 47.92| 41.31| 47.09| 43.93| 44.52| 30.10| 27.36| 36.34| 38.11|= SWA022 [ SWA027] 21|37.14|42.96|38.66|55.35|49.40| 45.68|47.68| 57.38|47.16|37.27| 22.10| 43.06|42.49|= SWA023 [ SWA027] 23|35.68| 47.46| 44.47| 53.73| 46.32| 44.06| 43.40| 45.33| $38.59|38.84| 25.99|40.29| 42.72 \mid=$ SWA024 [ SWA027] 23| 40.44| 54.98| $53.11 \mid$ 58.62| 47.26| 49.34| 39.90| 49.99| $43.28|42.45| 32.45|37.42| 54.65 \mid=$ SWA025 [ SWA027] 23| 43.61|64.20|62.58|61.06|61.71|57.21|56.95|63.53| $51.92|48.77| 36.65|54.38| 56.70 \mid=$ SWA026 [ SWA027] 21| 40.10| 45.60| 30.91| 46.94| 40.97| 39.76| 47.99| 56.90| 42.43| 33.90| $24.38|51.93| 36.10 \mid=$ SWA027 [ SWA027] 19| 42.05| 39.49| 40.89| 50.17| 47.16| 42.64| 47.30| 49.96| 41.10| 31.04| $26.42|41.42| 41.05 \mid=$ NAM001[ NAM034] 8| 12.70| 13.73| 15.69| 21.25| 13.71| 9.81| 14.85| 18.45| 10.19| 13.43| 12.35| 5.66| 15.17|= NAM002[ NAM034] 9| 10.08| 16.28| 14.63| 18.33| 7.56| 8.27|11.31| 13.21| 9.11| 8.53| 12.80| 6.18| 10.78|= NAMOO3[ NAMO34] 9| 14.38| 17.84| 18.07| 20.49| 11.83| 11.44| 16.56| 18.52| 10.18| 12.88| 14.94| 7.94| 15.11|= NAMOO4[ NAMO34] 9| 18.12| 18.80| 17.84| $24.90|17.96| 12.93|16.72| 23.92|14.37| 17.15|20.64| 6.32|20.66|=$ NAM005[ NAMO34] 9| 17.28| 15.83| 17.46| 24.18| 15.32| 12.62| 16.55| 17.31| 15.78| 15.30| 17.55| 6.97| 16.70|= NAMOO6[ NAMO34] 10| 15.13| 17.32| 15.93| 19.51| 13.85| 9.69| 10.01| 14.56| 11.50| 8.54| 13.84| 7.79| 13.67|= NAMOO7[ NAMO34] 9| 12.28| 20.34| 17.68| 19.04| 10.85| 12.58| 16.71| $20.70|13.60| 12.13|14.26| 6.92|13.96|=$ NAM008[ NAM034] 9| 15.41|17.82|16.12| 17.12|11.93| 7.88|11.04|17.16| 9.08| 13.34|15.24|5.96|13.19|= NAMOO9[ NAMO34] 9| 15.23| 18.15| 18.79|21.86| 14.81| 10.51| 13.57| 19.32| 11.14| 15.26| 16.20| 6.48| 17.68|= NAM010 [ NAMO34] 8| 12.23| 14.59| 17.87| 22.16| 13.11| 11.11| 12.46| 16.10| $11.25|10.02| 15.67|6.61| 16.49 \mid=$ NAM011[ NAMO34] 8| 14.65| 15.17| 18.51| 20.77| 13.35| 10.12| $15.13|17.28| 10.54|16.42| 13.53|5.96| 15.22 \mid=$ NAM012[ NAMO34] 9| 9.62| 13.99| 16.30| 20.72| 11.47| 9.72| 13.31| 15.54| 11.13| 8.85| 12.61| 5.65| 13.27|= NAM013[ NAM034] 9| 10.27| 15.31| 16.35| 18.04| 8.87| 8.87| 12.13| 12.86| 10.41| 8.34|11.99| 4.81|11.55|= NAM014[ NAMO34] 8| 15.08| 12.81| $15.46|19.58| 12.39|8.10| 10.94|13.84| 10.44|12.41| 12.37|5.71| 12.22 \mid=$ NAM015[ NAM034] 8| 13.47| 14.32| 14.91| 25.83| 15.14| 14.10| 19.23| $21.08|15.24| 14.98|15.13| 6.80|17.23|=$ NAM016[ NAMO34] 7| 15.13| 15.55| 16.84| 18.96| 10.22| 12.84| 11.14| 17.51|11.83| 10.74| 15.63| 8.13| 19.42|= NAM017[ NAMO34] 9| 22.40| 18.58| 22.71 | $26.23|12.83| 18.55|15.22| 19.15|16.38| 14.62|21.44| 8.46|22.54|=$ NAM018[ NAM034] 10| 16.61| 17.80| 17.00| 26.05| 14.06| 11.13| 11.11| 13.63| 12.70| 13.84| 15.71| 8.69| 15.04|= NAM019 [ NAMO34] 10| 20.71| 20.82| 20.47| 22.73| 12.55| 16.37| 10.98| 20.51| 14.43| 12.90| 18.30| 8.29| 22.01|= NAMO20 [ NAMO34] 10| 22.94| 21.88| 20.97| 24.17| 14.56| 16.86| 13.35| 19.80| 12.24| 13.79| 17.48| 7.78| 20.12|= NAM021[ NAM034] 9| 15.77| 17.36| 16.49| 19.27| 10.87| 8.65| 8.93| 15.81| 12.31| 9.91| 14.25| 6.49| 14.81|= NAMO22 [ NAMO34] 10| 20.99| 19.90| 19.46| 21.57 | 10.74 | $13.75|14.84| 19.44|15.31| 14.71|18.53| 7.28|16.60|=$ NAMO23 [ NAMO34] 10| 24.99| 19.80| 19.65|29.02| 14.87| 16.00| 14.71| 20.68| 13.13| 18.43| 21.62| 8.95|20.46|= NAMO24[ NAMO34] 8| 19.43| 20.40| 22.55| 22.05| 16.79| 13.47| 16.29| 19.18| 17.43| 13.10| 16.73| 7.11| 18.56|= NAMO25[ NAMO34] 9| 15.26| 16.34| 19.90| $22.32|14.50| 16.65|14.18| 17.32|14.22| 12.76|14.83| 7.90|20.86|=$ NAMO26[ NAM034] 9| 17.90| 18.90| 17.76| 24.28| 13.72| 12.84| 14.88| 19.26| 13.49| 15.93| 13.15| 9.88|19.59|= NAM027[ NAMO34] 9| 18.94| 19.35| 20.67| 27.12| 19.63| 13.75| 13.68| 19.80| 12.08| 15.73| 16.66| 6.13| 22.36|= NAMO28[ NAMO34] 7| 16.58| 15.43| 17.07| 23.71| 17.47| 13.00| 15.15| 16.17| 14.84| 14.07| 17.82| 7.92| 17.15|= NAMO29[ NAMO34] 9| 19.16| 21.74| 18.28| $25.62|14.84| 10.80|11.92| 15.46|15.39| 13.81|15.58| 9.17|17.21|=$ NAMO30 [ NAMO34] 9| 17.77| 18.22| 17.84| 25.57| 14.79| 13.92| 13.51|21.60| 11.81| 12.88| 15.74| 7.18| 20.70|= NAM031[ NAMO34] 9| 26.21| 19.11|24.26| 26.64| 16.57| 22.14| 18.48| 20.17| 20.12| 19.77| 23.09| 10.93| 24.66|= NAM032[ NAMO34] 9| 18.55| 20.14| 18.96| 26.38| 14.91| 13.89| 13.96| 17.68| 11.49| 12.78| 14.70| 5.21| 19.24|= NAM033[ NAMO34] 7| 16.18| 13.71| 17.47|21.10| 13.02| 9.50| 10.99| 13.54| 11.53| 10.45| 13.60| 4.03| 16.42|= NAM034[ NAM034] 7| 18.35| 20.88| 13.61| $25.67|14.64| 11.58|10.63| 12.65|9.6||18.13| 18.58|15.12| 18.42 \mid R S P 026$ DOR001 [ DORO23] 21|31.85| 43.81| 42.52| 56.93| 27.79| 38.52| 31.51| 35.62| 37.16| 37.56| 38.34| 35.77| $24.74 \mid=$ DOR002 [ DORO23] 22| 40.67| 45.07| 49.55| 52.67| 39.77| 45.42| 39.83| 49.28| 43.21| 39.65| 41.40| 38.85| 26.59|= DOR003 [ DORO23] 20| $30.45|37.88| 43.41|46.96| 34.85|28.41| 32.71|37.12| 37.65|27.85| 33.34|30.17| 25.60 \mid=$

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