GENETIC CHARACTERIZATION OF INDIGENOUS GOAT POPULATIONS OF MOZAMBIQUE

by

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Declaration

This is my original work and has been submitted to the University of Pretoria for the degree of Master Science.

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Dedication

To my daughters Stívellan and Kiemy Bule and my husband Joaquim Bule

In memory of my mother and my father

Acknowledgement

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Abbreviations

AFLP	Amplified Fragment Polymorphic DNA
AMOVA	Analysis of molecular variance
ARC- AII	Animal Improvement Institute – IRENE
BC	Before Christ
Bp	Base pairs
D*A*	Modified Cavalli-Sforza and Edwards' genetic distance
DNA	Deoxyribonucleic Acid
D*s*	Nei´s standard genetic distance
FAO	Food and Agricultural Organization of the United Nations
Fst	The amount of differentiation among subpopulations relative to the limiting amount under complete fixation
GDP	Gross Domestic Product
He	Expected heterozygosity
Ho	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
MNA	Mean number of alleles
MtDNA	Mitochondrial Deoxyribonucleic Acid
N	Number
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymerase DNA
RFLPs	Restriction Fragment Length Polymorphisms
PAGE	Polyacrylamide gel electrophoreses
SADC	Southern African Development Community
STRs	Simple Tandem Repeats
UPGMA	Unweighted Pair Group Method with Arithmetic mean
USA	United States of America

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Summary

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Genetic characterization of Mozambican goats was done using microsatellites markers. The genomic DNA from 160 unrelated animals from 4 provinces was extracted and PCR-amplified with a panel of 17 microsatellite markers. PCR amplifications were visualized using 5% polyacrylamide gel electrophoresis on an ABI 377 automated sequencer. The data was captured using Genescan 3.1 software and data analysis was carried out using Genotyper 2.0 to determine the fragment sizes in base pairs.

The microsatellites chosen in this study amplified well in goats. Allele frequencies ranged from 0.010 to 0.99 for any specific microsatellite. Alleles unique to certain populations were observed with Pafuri goats showing the highest number (13) with allele frequencies ranging from 0.013 to 0.307. The MNA ranged from 5.59 in the Tete population to 6.94 in the Pafuri population within all individuals. The observed heterozygosity (Ho) values ranged from 53% for the Maputo population to 59% for the Pafuri population. The average observed heterozygosity estimate for all populations was 56%. The genetic distance estimates of Nei (1972) were used and ranged from 0.037 to 0.205. The greatest genetic distance was observed between the Maputo and Pafuri

populations. The highest gene flow (8.36) was observed between the Tete and Maputo populations. 84.38% of populations studied were correctly assigned to their original population.

The results indicate that the Pafuri and Cabo Delgado populations are the most distinct within all the Mozambican goat populations. There is sufficient genetic variation within Mozambican goat populations with distinct genetic differentiation between the Cabo Delgado and Pafuri goats and the Maputo and Pafuri goats which suggests that they are really different breeds.

CHAPTER 1

LITERATURE REVIEW

1.1. The livestock revolution

Several contributors refer to a strongly demand-led "livestock revolution" that is taking place as the result of a rapidly growing world population, income growth, increasing urbanization, changes in lifestyles and food preferences. In addition, global drives for changes in certain livestock sectors include increasing consumer health concerns, the continuing growth of fast-food chains and the increasing consumption of convenience processed food (FAO publication, 2004).

In 1998 the total human population of sub-Saharan Africa was estimated to be 570 million, of which over 60% depended on agriculture as a source of income. This is compared to 76% in 1975 and 65% in 1994. However, projected population increases (estimated growth of 2.5% per annum), will intensify the demands made on African agriculture in the future. Vast areas of Africa are unsuitable for crop production (around 65%) especially around desert regions where only livestock are suitable for use in farming systems (FAO publication, 2000[a]).

Between 1995 and 2020, approximately 97.7% of global population increase will be in developing countries, by which time 84% of the world's people (an estimated 6.3 billion) will be living in developing nations. The demand for meat in the developing world will double by 2020. Between the mid 1970s and 1995, meat consumption in the developing world rose from 11 kg to 23 kg per person. Two major contributors were China and Brazil. If China and Brazil are excluded, the increase per person per year was from 11 kg to 15 kg *per capita* (FAO publication, 2004).

The global demand for meat is expected to grow by 57% from 208 million tons in 1997 to 326 tons in 2020, while the demand for meat in sub-Saharan Africa is expected to increase from 5.5 million metric tons to 11.2 million metric tons during the same period (Thairu – Muigai, 2002). This increase in demand for animal products, being termed the "Livestock Revolution", has already begun and has been brought about by changes in the diet of an emerging middle class in the developing countries (Delgado *et al*., 1998).

The global demand of poultry meat will increase by up to 85%, beef by 80% and pork by 45% by 2020. The growth of meat and milk consumption in the developing world is predicted to be 2.8% and 3.3% annually from 1990 and 2020, in marked contrast to 0.6% and 0.2% in developed countries (Delgado *et al*., 1998).

Consumption in the developing world is determined by purchasing power and the greater consumption of meat and milk will be stimulated by economic growth and more disposable income in the growing, more prosperous middle class. In order to meet this demand, there has to be a corresponding global increase in livestock production, especially in sub-Saharan Africa where this demand is anticipated. Part of the strategy to increase livestock production in sub-Saharan Africa should be brought about through the use of indigenous livestock breeds. For centuries indigenous livestock breeds have been providing the African people with food, clothing, draught power, manure and financial security as well as being used for various cultural, religious and recreational purposes (Thairu – Muigai, 2002).

The need to achieve food security is now greater than ever before. One out of six individuals in the world today does not have sufficient food and many people in sub-Saharan Africa suffer from a lack of sufficient calories or protein (FAO publication, 1999).

The basic food of African people such as maize and rice offers calories but are poor on micronutrients or protein. While it possible to obtain adequate protein from vegetables, it is then necessary to eat a wide variety, something the poor in Africa cannot do. Livestock

are an excellent source of high quality protein, minerals, vitamins and micronutrients for the African people, especially in semi-arid and tsetse-fly infested areas where exotic livestock breeds cannot survive (FAO publication, 2000[a]).

Livestock contribute to food security by providing meat, milk, blood and meat and milk products. The contribution of animal production to the global economic value of food and agriculture has been estimated to be between 25 and 35% to the Gross Domestic Product (GDP) (Thairu – Muigai, 2002). This is an indicator of the importance of livestock to national economies.

Meat, milk, hides, wool, manure and fees charged for draught power make essential contributions in sub-Saharan Africa at a household level. It has been estimated that up to 1.96 billion people rely on livestock to supply part of or their entire daily needs. There is, consequently, the need for conservation and the sustainable use of the indigenous livestock genetic resources that are found on the Africa continent (FAO publication, 2000[a]).

1.2. The indigenous animal genetic resources of sub-Saharan Africa

Sub-Saharan Africa in particular is an important source of animal genetic resources with a wealth of domesticated animal diversity. This has been generated in response to the variety of challenges faced by animals, primarily the wide range of agro-ecological zones and the considerable number of endemic diseases. These factors, coupled with frequent famines and political instability, place a range of demands on livestock, which cannot be met by any one breed or even by a small number of breeds (FAO publication, 2000[b]).

The estimation of total number of species of living organisms on earth has varied from 2 million to 100 million, with best estimates of approximately 10 million species, of which only 1.4 million have been classified (FAO publication, 2000 [b]). From this, the FAO has

estimated that there are slightly over 40 domestic livestock species of which approximately 30, represented by an estimated 4,500 breeds/strains, have indigenous representation in Africa (Thairu – Muigai, 2002).

In 1995, 317 mammalian and avian breeds were recorded in the African region in the global DataBank for Farm Animal Genetic Resources. Since then, one avian and 315 mammalian breeds have been added, increasing the amount of data recorded for the African region by 1% and 99% respectively to give a total of 738 breeds. Surprisingly, in the Databank for Farm Animal Genetic Resources where the risk status of mammalian and avian breeds recorded for each species in Africa region up to 1995 and up to 1999 are shown, only 14% (95 of 699) of existing breeds are categorized as at risk. This is believed to be a gross underestimate of the actual situation, primarily due to a lack of information (Sherf, 2000).

Despite such biases, when the complete data sets are indirectly compared, some trends are clear. As the percentage of the total number of existing breeds that have population data (and therefore the risk status is known), the number of mammalian breeds recorded in the African region at risk of extinction has increased from 8% (of 179) to 19% (of 388) since 1995. The Pafuri goat of Mozambique is included in this list. This figure is alarming and efforts must be made to encourage the maintenance of these domestic animals where the genetic resource is at risk (FAO publication, 2000 [b]).

1.3. Replacement of lost animal diversity

Domestic animal diversity cannot be replaced. As much as novel biotechnologies may attempt to improve breeds, it is not possible to replace lost diversity particularly over the time horizon now required to meet the human induced imperative. In practice, loss of diversity is forever. Biotechnology offers the opportunity to better characterize, utilize, conserve and access animal genetic resources for food and agriculture production. However, there is neither an existing technology nor is there likely to be a future

biotechnology with the capacity to create and equal the naturally occurring diversity in the world today. Providing that the inherent diversity associated with the farm animal species is conserved as a store of genetic potential, changes and improvements to existing breeds will continue to occur naturally over time in response to the various dynamic environments, humankind's changing needs and through genetic drift (Sherf, 2000).

To date, only a small number of engineered genes have proven useful for the improvement of plant production. Some transgenic cultivars of major food crops incorporating resistance to stress factors such as temperature, pests and herbicides and with the potential to produce added food supplements have been successfully produced. The use and distribution of such plants is increasing rapidly. Animals, however, are more complex and costly than plants. All animals contain about 80 000 genes all of which interact in a complex system with each other (Weller, 2001 & FAO publication, 2000 [b]).

Unique combinations of genes are responsible for the adaptive fitness of a breed necessary for production in a particular environment. Transgenic alterations to individual genes are now becoming possible. In the near future these will possibly begin to supplement the classical selective breeding practices offering added opportunities to realize food security. The potential risks in doing this will need to be assessed on a case-by-case basis against the benefits of achieving a more rapid genetic improvement in food and agricultural production (FAO publication, 2000 [b]).

The management cost required in maintaining the existing pool of animal genetic diversity in such a way as to protect and prepare for a range of indeterminate, unforeseeable future uses are, however, negligible compared to the massive cost involved in biotechnology development. In addition, although biotechnology can contribute to agricultural improvement and aid conservation efforts, in no way does it have the capacity to generate diversity if it is lost. For developing countries, the practice of good management of their treasure chests of genetic potential remains the most viable option and essential to ensure the future sustainability of animal production for agriculture (Sherf, 2000).

1.4. The origin of domesticated goats

1.4.1. Distribution and evolution of goats

Because of the goat's undoubted antiquity and its present distribution over a large part of the habitable globe, it is pertinent to inquire into its ancestry and probable evolution. Archaeological evidence suggests that it has been associated with man for up to 10 000 years. Although it is encountered today over a wider geographical area than any other domesticated farm animal, much confusion has arisen in attempts at its classification (French, 1980). Goats belong to the tribe Caprini of the family Bovidae of the hollow-horned ruminants in the suborder Ruminantia in the mammalian order of Artiodactyla (French, 1980 & Zeuner, 1963).

Much has been written and surmised on the origins of goats but there is inadequate factual information on which to develop any final conclusion. Although it was suggested that the Caprini type developed from a Miocene ancestral type, the earliest fossil evidence of a goat-like animal, the Tossunoria, is recorded from the Lower Pliocene in eastern China. Goat remains found in Europe and Asia indicate that, by Pleistocene times, these animals had became much more common and more closely related to certain species of Capra and Hamitragus which are still in existence today (French, 1980).

The tribe caprini is comprised of five genera. Two of these, *Capra* and *Hemitragus*, are true goats; one genus, *Ovis* is the sheep; and two genera: *Ammotragus* and *Pseudois* are goat-like sheep or sheep-like goats. According to latest taxonomic opinion the two genera of true goats are divided into three species of *Hemitragus* or Tahrs and six of *Capra*. All Tahrs have the same chromosome number (2n=48). Most of the *Capra* species are interfertile although for some pairs no crosses are recorded; all the species examined (*Bezoar, Ibex* and *Markhor*) have the same number of chromosome (2n=60) (Payne & Wilson, 1999).

Existing European goats have been fairly well described, documented and classified but this does not permit an understanding of their origins prior to their appearance in Europe.

Far fewer breeds and types have been reported from among the many different kind of goats found in Africa, Asia and only a relatively small number of breeds from these continents have been adequately differentiated and characterized, largely because they have been insufficiently studied by zoologists (French, 1980).

1.4.2. The wild goat

This is one of several classifications of wild goats and the suggestion that one can write authoritatively on the origin of the goat would be foolish because differences of opinion still exist on this subject despite considerable speculation by scientists (French, 1980).

A number of subspecies of wild goat is still encountered in the Pyrenees and can be traced from there, through the Alps and mountainous regions of eastern Europe, to the Caucasus and onward along the higher ground of the near East to the Himalayas and the central Asian mountain massifs. Offshoots branch away from this Caprine chain to the wild goat encountered in Ethiopia, southern Arabia and southern India (French, 1980).

The wild goats that still exist include the Turs *C. caucasica* which somewhat resemble the goat-like Ammotragus sheep. These heavy animals have big horns, which are almost circular in section and curve outwards, upwards and backwards. They are found only in the Caucasus Mountains (French, 1980).

The ibex group is more varied and more widely distributed. There are two species and nine subspecies of ibex to be found in Europe, Asia and Africa. The Spanish ibex, *C. pyrenaica,* has a horn form similar to the Trus, with which group it has sometimes been included.

The sharp inner edge of the horn keel gives the horns a rather triangular cross section, the front edge of which is typically knotted by cross ridges (French, 1980).

The four subspecies of this particular wild goat are found in the Iberian Peninsula. The other species, *C. ibex*, has long, scimitar-shaped horns that curve backwards and have well marked cross ridges on their flat front edge. The five subspecies of *C. ibex* are the Alpine ibex with small ears and beard, the Caucasian ibex which is rather similar to the Turs, the Siberian ibex which is the largest and most widespread of the group and has big horns and a large beard, the Nubian ibex which has long, slender horns curving backwards, long ears and a long beard and the Abyssinian ibex that has a shorter beard and thicker horns than the Nubian subspecies. Unlike other wild goats, the Nubian goat has a convex facial profile (French, 1980).

The Bezoars or Pesangs are wild goats with horns, which rise vertically from the head and then arch backwards in a long, scimitar-shaped curve. Their horns have a sharp anterior keel instead of the broad, ridge surface of the preceding species. They constitute the *C. hircus* species in which there are two subspecies, the Persian and Sind wild goats (French, 1980).

The large Markhor goats are heavily built and have a big beard. Their horns twist upwards and backwards in a spiral and have sharp anterior and posterior keels. This *C. falconeri* comprises seven subspecies, recognizable by the twist of the horns, which varies from a curved shape with an open spiral to a nearly straight form with a screw-like spiral. The Markhors are encountered in mountains from Afganistan to Kashmir (French, 1980).

The three remaining species of wild goat, which constitute the Tahr group, belong to the genus *Hemitagrus*. This group differs from the *Capra* genus by its characteristic shorter and thicker horns, which sweep backwards in a smooth curve and are only slightly longer than the head. The males of the Tahr group have no beards and the hair is rather long and shaggy. The three subspecies are the Himialayan, the Nilgiri and the Arabian Tahrs, the latter being the smallest of the known goats (French, 1980).

1.4.3. Domestication of goats

Goats were almost certainly the first ruminants to be domesticated (Devendra & Mcleroy, 1982) and were possibly the second species to be taken into the humanfold after the dog (Zeuner, 1963). South-West Asia (Iran & Iraq) is the most likely origin of the domesticated species of the bezoar, *C. aegagrus* (Payne & Wilson, 1999).

Although not certain, the available evidence from comparative morphology and breeding experiments indicates that the bezoar of western Asia is the main ancestor of most domestic goats (Devendra & Mcleroy, 1982).

Archaeological evidence indicates that goats, in the form of their wild progenitor the bezoar (*C. aergagrus*), were the first wild herbivores to be domesticated (Fig.1.1). These studies suggest that this occurred approximately 10,000 years ago at the dawn of the Nelotic in the region known as the Fertile Crescent. Zeder & Hesse (2000) confirmed that the fertile crescent region of the Near East was the centre of domestication for a remarkable array of today's primary agricultural crops and livestock. Wheat, barley, rye, lentils, sheep, goats and pigs were all originally brought under human control in the broad area that stretches from the southern Levant through south eastern Turkey and northern Syria, to the high Zagros mountain pastures and arid lowland plains of Iraq and Iran. For more than 50 years researchers have sought to define the sequence, temporal placement, and social and environmental context of domestication. They described recent research that uses a study of modern wild goat *C. hircus aegagrus* to develop an unequivocal marker of early goat domestication, which we apply to assemblages that lies both within and outside the natural range of wild goats in the eastern fertile crescent region, long thought to be the initial heartland of goat domestication (MacHugh & Bradley, 2001).

Luikart and colleagues add *C. hircus* to the growing list of domestic animals that have been widely surveyed for mtDNA sequence variation. In their survey, they demonstrate that the structure and distribution of mtDNA variation in domestic goats are qualitatively

different from the patterns observed in other large Eurasian herbivores domesticated for food, skins and fibre (cattle, buffalo, pigs, and sheep) (MacHugh & Bradley, 2001).

Figure 1.1: A third or fourth century BC Mesopotamian stone carving of a man carrying either a domestic or a wild goat. This piece is displayed in the Louvre Museum in Paris. (Figure courtesy of Mike Schwartz.) Extracted from MacHugh & Bradley, 2001.

It is assumed that the goat would have been initially more useful ecologically to Nelotothic farmers since its browsing behaviour would aid in clearing forest, after which sheep would have emerged as the economically superior animal (Thairu-Muigai, 2002). These sturdy animals may have been the first "Walking lards" and, for example, they could have triggered the subsequent domestication of the full repertoire of Eurasian livestock species that have provided the bulk of the animal protein consumed by ever-expanding human populations (MacHugh & Bradley, 2001).

Domestication occurred gradually over a period beginning some 11 000 years BC (before present) and it probably first spread to central and south eastern Asia. By 5500 BC goats had migrated into sub-Saharan Africa and a dwarf type was recorded from that period near Khartoum in the Sudan. Initially, the migration routes of the human population may have promoted the expansion of domestic goats and their establishment in several regions. Many breeds are adjusted to the climate, diseases and nutritional conditions and, consequently, developed the capacity to survive and reproduce in difficult conditions. In

addition they developed a great aptitude to increase production without losing local adaptation through selection programmes (Payne & Wilson, 1999).

In Africa south of the Zambezi River, goats were introduced shortly before and after the arrival of European settlers. The goat population is, therefore, derived from various breeds that were brought from Bantu tribes in the north. On the other hand, the population has been influenced by the Boer type goat, which was developed from the 18th century onwards (Gall, 1996).

1.5. Genetic resource of goats in the Tropical Africa and Mozambique

The goat population of the world comprises of approximately 674 million, of which 94% are found in developing countries (FAO publication, 1996). Africa and Asia account for about 81% of the total population. The annual growth rate of 3.3% is considered the highest in comparison to other ruminants. In Africa, these animals are concentrated in Nigeria, Ethiopia, Sudan and Somalia. From a world total of 351 goat breeds, there are approximately 146 in Asia, and 59 in Africa (Devendra, 1998).

In most of the developing countries goats play an important role in the sustenance of rural families and contribute significantly to supplying their needs in animal proteins. This is due to the capacity of goats to convert low quality grazing into useful products for humans, such as meat, milk, coats, manure, etc. A great advantage is their low production cost, short reproduction cycle and their small size, which facilitates the slaughter and consumption of the meat by a family without the risk of deterioration, taking into account the absence of refrigeration facilities in the rural areas (Gall, 1981; MacHugh & Bradley, 2001).

Tropical Africa contains one third of all the world's goats. On average there is one goat on every 10 ha of tropical Africa and there is 1.1 head of goat per person employed in the

agricultural sector. Goats and sheep are equivalent in weight terms to about 17% of the total domestic ruminant biomass of tropical Africa (Payne & Wilson, 1999).

The total meat production from African goats and sheep combined is estimated at 1.15 millions tons which is equivalent to about 16% of the total world output from these species. Milk from small ruminants is about 14% of world production. Goats in tropical Africa are much more important than sheep as milk producers and they are estimated to produce about three times as much milk as ewes. Small ruminant skins from Africa, estimated at 258 000 tons, represent about 16% of world production, the proportion from goats at 25% being much greater than from sheep (FAO publication, 1985).

Livestock and agriculture in Mozambique are major sources of income and employment for over 85% of the population who dwell in the rural areas (Maciel *et al*., 2004). Livestock itself contributes 5% to national economy. However the consumption in 1997 was approximately 12.5 kg of meat, 2.4 l of milk and one egg *per capita*. When compared with the consumption in Africa, these values are low (13 kg of meat, 30 l of milk *per capita*) (Conselho de Ministros Report, 1997). Goats are generally used for meat production and provide extra earnings to smallholder farmers in these rural areas. In addition, goats are slaughtered on festive occasions and used for traditional ceremonies and “lobolo”, and sometimes for milk (Morgado, 2000).

1.5.1. The indigenous goat genetic resources of Africa

Indigenous goats have been classified into two main groups, the long-eared and short-eared (Gall, 1996). This is not a particularly useful system and, more appropriately, others describe goats as large, small or dwarf type (table1). Large types, which may also have disproportionately long legs, are found along the southern fringe of the Sahara and in southern Africa. The small type is mainly distributed in eastern Africa and the dwarf types, which are also to some extent tolerance of trypanosomiasis, are found mainly in humid western Africa. Dwarf types are usually more prolific than small and large types (FAO publication, 1985).

Table1.1 - Types and distribution of some African goats

Type of goats	Distribution	Example of breeds
Large goats	Southern fringe of Sahara and in Southern Africa	Africander, Pafuri, Tswana, Swazi, Ndebele, Landim, Shukria, Sudan Desert, West African Long-Legged.
Small goats	Eastern Africa	Red Sokoto, Afar, Mubende, Kigezi, Boran, Masai, Rwanda and Burind, Malawi, Zimbabwe (Mashona).
Dwarf goats	Western Africa	West African dwarf

In FAO 1985

1.5.2. The indigenous goat genetic resources of Mozambique

Livestock and agriculture in Mozambique are major sources of income and employment for over 85% of the population who dwell in the rural areas. Goats are generally used for meat production and provide extra earnings to smallholder farmers, who hold more than 95% of the national herd. In addition, goats are slaughtered for festive occasions and used for traditional ceremonies and "lobolo" (Morgado, 2000). The Pafuri is probably the only breed milked for home consumption, especially when food resources are scarce (Maciel *et al.*, 2004).

The classification of goat types has traditionally been based on characteristic morphological features such as horns, colour, hair, ears, size, and weight. Two main types are found in Mozambique.

The 'Landim' breed is the major goat breed distributed throughout the country with little variation in size and adaptation. It is mainly found in semi-arid to sub-humid monomodal rainfall areas in the southern regions of the country and is also called the Portuguese Landrace (Gall, 1996). The Landim from Tete Province appears smaller than in the south

of Mozambique but has a higher fertility rate than in the south (Maciel *et al.*, 2004). According to these authors it would be interesting to characterize and to study the performance of this breed in that province.

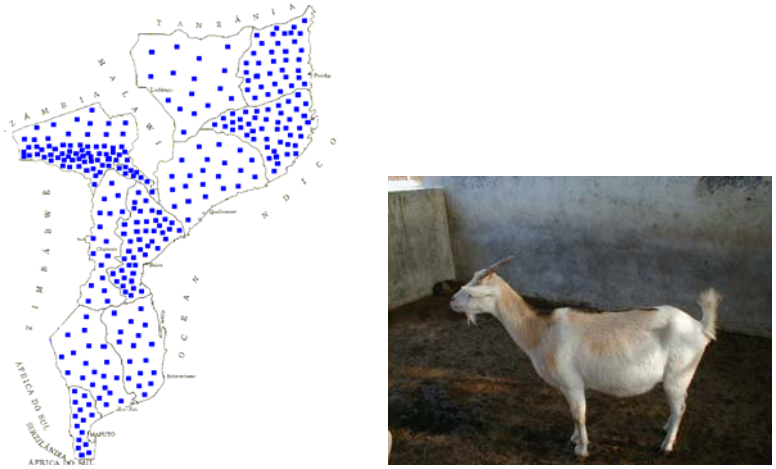

Figure 1.2. Map of distribution of Landim goat and photograph

The 'Pafuri' breed is a result from crossing Boer goat males with Landim females. The Boergoats were introduced from the northern Transvaal in 1928 into a small area in south-west Mozambique known as Pafuri. The breed is kept under an agro-pastoral to pastoral management system generally in a semi-arid to arid environment (Gall, 1996). It is very typical of the transhumance system in the Gaza Province where it is milked during the drier periods of the year. Nevertheless, its occurrence is spreading to other south provinces, such as Maputo and Gaza (Maciel *et al.*, 2004).

Figure 1.3. Map of Mozambique and distribution of Pafuri goat and photograph

1.6. Genetic Identification of breeds and measuring genetic variability

Very limited information on the genetic variability measurements and genetic differences of Mozambique goat breeds exists. It is unclear as to whether different breeds exist or whether only different ecotypes or populations can be identified according to the areas where they occur. Such information will contribute to the preservation of the local breeds as an investment guaranteeing the potential use in future breeding programmes.

The term 'breed' is not well defined and creates a problem in the African context. The animal-orientated definition recognizes that breeds differ by the totality of average differences observed in many quantitative and qualitative traits. The differences may overlap but they have a genetic basis and these differences taken together provide a unique description. This definition provides a solid basis for the application of population genetic techniques and is in stark contrast to the arbitrary and often colloquial designation of a particular type to one breed or another based on anthropocentric criteria (Meghen *et al.*, www.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm - 32k).

The use of a genetic study to determine the genetic make-up of the breeds or populations in Mozambique will contribute to information and the better understanding of goat genetic resources. The genetic characterization of these breeds would be a powerful tool for breed conservation and improvement.

Several techniques have been developed to estimate the genetic variation or polymorphisms in populations and, hence, the genetic relationship amongst populations. Some major techniques with practical application will be discussed.

1.6.1. Polymorphic genetic techniques

Protein and isozyme polymorphisms

This technique was widely used during the 1980s – 1990's in population genetics. Research has been carried out worldwide using blood biochemical polymorphisms in order to study the genetic relationship between populations (Kidd, 1974; Tuñón, Gonzalez &Vallejo, 1989; Casati *et al.,* 1999; Kotzé *et al.*, 2000). Protein polymorphisms have proved to be a cheap and fast method of analysing single locus variation in breeds (Thairu-Muigai, 2002). Protein polymorphism studies, however, are now of limited value in the assessment of genetic variation as they detect relatively low levels of polymorphism, resulting in a lower resolving power for genetic characterization studies (Meghen *et al.*, www.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm-32k).

Restriction Fragment Length Polymorphisms (RFLPs)

This technique relies on the amplification of variable regions of the target genome, with amplicons then being digested with one or more sequence-specific restriction enzymes. The DNA fragments of different lengths are then subjected to electrophoresis and fragments migrate according to their weights, the smaller fragments faster and the large fragments more slowly. Thus, RFLP generally refers to the differences in banding patterns obtained from DNA fragments, after sequence-specific cleavage with restriction enzymes (Van Marle-Köster, 2003). This technique can be applied to nuclear DNA or to mitochondrial DNA (also to chloroplast DNA in the case of plants). It has applications in the study of genetic distances, genetic variation, gene flow, effective population size, patterns of historical biogeography and analyses of parentage and relatedness. Since mutational events are generally the product of base substitutions, however, the rate of mutation is likely to be extremely low (10-7 to 10-8 per generation), and this results in a similar problem to that of proteins, which is, a lack of resolving power when dealing with very closely related groups. This has been demonstrated by Theilmann *et al.,* (1989), who

carried out a study of nine RFLPs in six breeds of cattle where only Brahman (*Bos indicus*) cattle differed significantly from the *Bos taurus* breeds (Meghen *et al.*, www.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm - 32k).

Random Amplified Polymorphic DNA (RAPDs)

RAPDs are known as arbitrarily primed Polymerase Chain Reaction (PCR) (AP-PCR), or as a DNA Amplification Fingerprinting technique (DAF). This technique is based on the use of short, arbitrary primers in a PCR reaction and can be used to produce relatively detailed and complex DNA profiles for detecting amplified fragments between organisms. In the simplest format, only one short oligonucleotide consisting of eight to ten nucleotides in length is used. However, multiple primers are usually applied and a range of five to 21 nucleotides has proven successful if detection is coupled with polyacrylamide gel electrophoresis. Relaxed PCR conditions allow for multiple unspecific priming sites on opposite DNA strands, even if the match is imperfect. A successfully amplified template sequence will, however, only span from a priming site sequence to a nearby complementary sequence. Depending on the primer template combination and ratios, amplified products range from less than ten to over a 100. In this way, a spectrum of products characteristic for each template and primer combination is typically obtained and these can be adequately resolved and visualized using polycrylamide gel electrophoresis and silver staining. Agarose gel electrophoresis and ethidium bromide staining can also be used to detect only the major fragments (Van Marle-Köster, 2002).

RAPDs have the advantage that they can be obtained at a reasonable cost and will generally amplify a range of fragments of most DNA and show polymorphisms. Certain primers will produce unrelated patterns between unrelated animals and identical ones for very closely related animals. Presumably primer sites are randomly distributed along the target genome and flank both conserved and highly variable regions. Wide variation in band intensity can be shown to be reproducible between experiments, which could be the result of multiple copies of the amplified regions in the template or the efficiency with

which particular regions are amplified. The polymorphic bands obtained from RAPDs can also be cloned for further analysis.

A major disadvantage is that the RAPDs are very sensitive to PCR conditions and this may lead to poor reproducibility (Van Marle-Köster, 2003). The consistency of results is not guaranteed as minor differences in experimental conditions can produce erratic results. Even under carefully controlled conditions, there can be ambiguity in the scoring of bands separated on a gel. Full understanding of the manner in which the genetic variation observed is generated and the reconstruction of evolutionary histories is difficult. RAPDs are dominant markers and heterozygosity can be scored as homozygosity which affects the accuracy of the information content (Meghen *et al.*, *w*ww.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm - 32k). In comparison to other genetic profiling techniques described in this study, the reliability of RAPDs is regarded as moderate (Van Marle-Köster, 2003).

Mitochondrial DNA

In animal cells, DNA is also found outside the nucleus in the mitochondria. Animal mitochondrial DNA can be easily isolated. It evolves five to ten times more rapidly than nuclear DNA and, a particular region, the D-loop, evolves even faster and is maternally inherited. Thus, mitochondrial phylogeny offers a relatively clear picture of the evolutionary history of a single genetic element. This strictly maternal inheritance of mitochondrial DNA can cause misinterpretation of the data and, consequently, the misreading of resultant phylogenies (Meghen *et al.*, www.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm - 32k). Some mitochondrial DNA studies have been performed on goats, for example the study by Sultana and Tsuji (2003) on Pakistan goats.

Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism is a DNA fingerprinting technique that is based on the detection of DNA fragments, subjected to restriction enzymes, followed by selective PCR amplification. The DNA is cut with two restriction enzymes and double

stranded adapters are then ligated to the ends of the DNA fragments to generate the template PCR. The specific adapter, ligated to the DNA fragment, determines the distribution of DNA restriction sites throughout the genome in question by DNA amplification. AFLP procedures can be manipulated to suit specific applications through the selection of the restriction enzymes and the design of the PCR primers. Typically, a rare-cutter restriction enzyme is combined to ensure the generation of small fragments (frequent-cutter) but to limit the number of fragments (rare-cutter) at the same time. PCR primers can be designed to have no selective bases on the 3´ends if the targeted templates are simple elements such as plasmids or bacterial artificial chromosomes. As in other techniques for fingerprinting, fragments are separated and analysed using gel electrophoresis. The AFLP technique can be performed at a reasonable cost, development costs are low but running costs are higher than for RAPD analysis but have the advantage of a higher reproducibility than RAPDs (Van Marle-Köster, 2003). This technique has also found application in limited genetic diversity studies of goats (Ajmone-Marsan *et al*., 2001; Crepaldi *et al*., 2001).

Microsatellites

Simple Tandem Repeats (STRs), or microsatellites, are a relatively new class of genetic marker. Microsatellites consist of tandem repeats of very short nucleotide motifs from one to six base pairs long, the dinucleotide repeat CA being the most common in mammalian genomes. A typical microsatellite locus may consist of a stretch of DNA with the base sequence CA repeated 12 times, i.e. $(CA)_{12}$. When the unique sequence flanking both ends of the repeated sequence is known, the microsatellite can be preferentially amplified using PCR. Different length classes (alleles) vary in the number of repeats and can be separated using polyacrylamide gel electrophoresis (PAGE). This class of marker is highly polymorphic by displaying many different alleles for a given locus (Meghen *et al.*, www.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm - 32k). It is not uncommon to find up to ten alleles per locus and heterozygosity values of 60% in a relatively small number of samples (Goldstein & Polack, 1997).

Microsatellites tend to mutate with mutation rates up to 10-2 per generation (Van Marle-Köster, 2003). This means that, although new length classes are generated at a rate fast enough to allow for the distinction of breeds, the rate is not so fast that relationships are obscured by homoplasy (identity of alleles as a result of separate mutation events as opposed to common ancestry) (Meghen *et al.,* www.fao.org/ag/AGa/AGAP/FRG/ FEEDback/War/t1300b/t1300b0j.htm - 32k).

A large number of microsatellite markers have been listed for various species that include cattle, horses, swine, sheep, goats, chickens, ducks, buffaloes, donkeys and camelids (FAO publication on Secondary guidelines, 2004). These markers are well dispersed through the genome and are applied in studies on genetic variability, parentage verification and genome mapping projects ((Zamorano *et al*., 1998; Saitbekova *et al.,* 1999, Gustavo *et al*., 2000; Martinez *et al*., 2000; Ritz *et al*., 2000; Mburu *et al*., 2003; Li *et al.*, 2002; Van Marle-Köster, 2003). There are public-domain databases of accumulated sequence data, such as GenBank and EMBL (ISAG/FAO, 2004; Meghen *et al.*, www.fao.org/ag/AGa/AGAP/FRG/FEEDback/War/t1300b/t1300b0j.htm - 32k).

Several studies using microsatellites specifically for goats have been conducted on Spanish, Asian, French, Italian, Chinese, Pakistan, Namibian, South African, Indian and sub-Saharan goats determining the genetic relationships between and among populations, genetic variation estimates and genetic diversity between populations (Tuñón, *et al.*, 1989; Luikart *et al.*, 1999; Yang *et al*., 1999; Ajmone-Marsan *et al*., 2001; Watts, Saitbekova *et al., 1999*; Barker, *et al*., 2001; Chenyambuga, 2002; Kim *et al.*, 2002; Li *et al.*, 2002; Sultana & Tsuji 2003; Kotzé *et al.*, 2004, Els *et al*., 2004; Martinez *et al.,* 2004; Visser *et al.*, 2004 & Tilagan *et al.*, 2006).

The United Nation's Food and Agriculture Organization on Farm Animal Genetic Resources published a document that evaluated the current status of molecular genetics research in different domestic animals (FAO Publication, 2004). This document concluded that microsatellite loci were the preferred marker for molecular genetic studies worldwide. Microsatellite data were applied in 66% of all genetic distance studies.

Biochemical markers were the second most frequently applied technique with a representation of 34% in studies.

For the effective utilization of indigenous Mozambican goat genetic resources, it is necessary to genetically characterize the different populations. Such characterization would provide a database with information on the genetic variation between and among the goat populations in the country. It would also provide information as to which of the populations represent homogeneous breeds and which are genetically distinct. Further information will contribute to the determination of the risk status of the populations and breeds. Ultimately, the information would contribute to the understanding of the evolutionary history of goats in Mozambique as well as to the future conservation and management of goat genetic resources.

1.6.2. Statistical Analysis

Hardy-Weinberg Equilibrium (HWE)

In a large random-mating population with no selection, mutation or migration, the frequencies and the genotype frequencies are constant from generation to generation and, furthermore, there is a simple relationship between the gene frequencies and genotype frequencies. A population with constant gene and genotype frequencies is said to be in Hardy-Weinberg equilibrium (HWE) (Falconer, 1989).

Factors that effect the HWE (Falconer, 1989):

- Mutation: This is the process that produces a gene or chromosome that is different from the wild type. By producing novel variants of genes, mutation brings about genetic variation in a population.
- Migration: This is the permanent movement of genes from one place to another. The migration of genes into a population results in an increase in that population's genetic variation and the migration of genes out of a population may result in a reduction in genetic variation.
- Non-random mating: It is possible to occur where related individuals have a greater probability of mating with each other than with other members of the

population and where individuals that are geographically close are more likely to mate with each than those are not as geographically close.

- Random genetic drift: The effect of genetic drift is infinitely proportional to the population size. When the population size is small, e.g. strong bottleneck effects in the past, there are greater changes in gene frequency under genetic drift at every generation. The smaller the population the greater the chances of sampling errors that occur.
- Selection: Only populations that are better adapted to the environment or are able to mate successfully are able to pass their genes on to the next generation. Selection generally results in a reduction of genetic variation in a population.

In this study, as in many studies of populations that have been genotyped, it was important to determine whether the loci and population genotyped were in HWE and whether there were any significant deviations from HWE. In this case, deviations of locus/population combinations from HWE were determined using GENEPOP 3.3 (Raymond & Rousset, 1995), POPGENE (Yeh, 1999) and GENETIX 4.0.2 programs.

Linkage disequilibrium

A population is said 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. Linkage disequilibrium analysis among pairs of loci in each population was done using POPGEN (Yeh, 1999) and GENETIX 4.0.2 computer programmes.

Genetic diversity

The mean number of alleles (MNA) detected in each population and the expected heterozygosities are good indicators of genetic polymorphism within populations. The MNA is the average number of alleles observed in a population, while the expected heterozygosities are the proportion of heterozygosities observed in a population.

MNA depends on sample size because of the presence of unique alleles in a population that occur in low frequencies. The number of observed alleles tends to increase with an increase in population size. The sample size of a population should be more or less the same otherwise the comparison might not be meaningful – random sample size of 20.

Genetic diversity can also be measured by calculating the average heterozygosity, which is the expected heterozygosity in a population that is assumed to be in HWE. Expected and observed heterozygosities were calculated using POPOGENE (Yeh, 1999) and GENETIX 4.0.2 computer programmes.

Genetic relationships between the breeds

Genetic distances

The genetic relationship between populations can be measured by determining the genetic distance between populations. This difference measured between two populations provides a good estimate of how divergent they are genetically.

When the genetic distance is large, the genetic similarity is high and the time they diverged from each other is smaller (Thairu-Muigai, 2002).

One of the common measurements of genetic distance in use today is Nei`s standard genetic distance (D_S) (Nei, 1972) whose value is proportional to evolutionary time when the effects of mutations and genetic drift are taken into consideration. However, Nei (1983) noted that the modified Cavalli-Sforza and Edwards' distance measure (D_A) is more efficient in determining the true topology of an evolutionary tree's being constructed using allele frequency data, especially if the populations are closely related. D_A has also been reported to increase more slowly with time and maintain a linear relationship for longer periods of time (Nei, 1983; Thairu-Muigai, 2002).

Construction of phylogenies

Phylogenetic analysis of populations has become an important tool for studying the evolutionary relationship of populations. It offers a simple graphic aid for visualizing the relationship between the populations, hence making interferences on the evolutionary histories easier (Thairu-Muigai, 2002).

The phylogenetic relationship of populations that were under investigation in this study was constructed using the neighbour-joining method in DISPAN to construct a phylogenetic tree from D_A and D_S distance measurements (TREEVIEW) Bootstrap test with 1000 replicates (Ota, 1993).

Genetic differentiation

The understanding of genetic structuring or differentiation within a population is of interest to geneticists because it reflects the number of alleles exchanged between populations that influence the genetic composition of individuals within these populations. Gene flow between populations determines the effect of selection and genetic drift generates new polymorphisms and increases the local effective population size. The Fst and Coefficient for genetic differentiation (Gst) are very commonly used to describe population differentiation (Thairu-Muigai, 2002).

Fst

Fixation index (Fst) is used to account for inbreeding within samples.

Fst is defined as the correlation between two alleles chosen at random within sub-populations relative to alleles sampled at random from total population. Therefore Fst measures inbreeding due to the correlation among alleles because they are found in same sub-population (Thairu-Muigai, 2002). Fst can be defined as:

Fst = (Ht – Hs)/Hs where Ht is the expected heterozygosity and Hs is the observed within population heterozygosity. Fst was calculated using POPGENE (Raymond and Rousset, 1995) and GENETIX 4.0.2 computer programmes.

Assignment of individuals to populations

In population genetics, individuals in a sample have to be classified into specific populations or breeds using a set of phenotypic criteria. The population assignment method assigns individuals using cluster methods. Individuals that are similar are assigned to the same cluster.

1.7. Aim and Objectives

The aim of the study is to genetically characterize different Mozambican goat populations using microsatellite markers to contribute to the regional (SADC) and FAO global database.

The objectives include the determination of:

1. the genetic relationships between the Mozambican goat populations
2. the genetic diversity within and between the Mozambican goat populations

CHAPTER 2

MATERIAL AND METHODS

2.1. Population Sampling

The study was carried out in four provinces of Mozambique, namely Maputo and Gaza in the southern region, Tete in the central region and Cabo Delgado in the northern region of the country. Figure 3.1 illustrates the Mozambican map with areas where samples were collected.

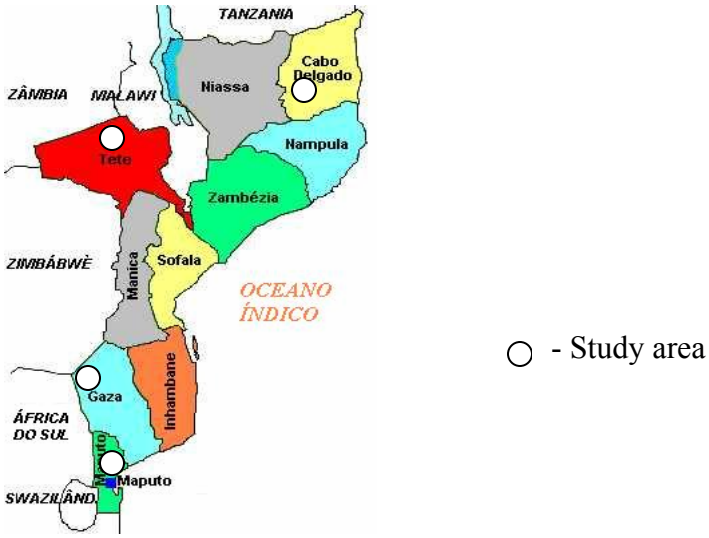

Figure. 2.1: Map of Mozambique indicating sample collection areas.

Forty unrelated animals were sampled from each population (10 males and 30 females) in the different provinces as recommended by ISAG/FAO, 2004. To ensure that individuals sampled were not closely related, different flocks were identified within the districts within each province (Table 2.1).

Hair with visible roots, at least 30+, were plucked from the tail of each animal and placed in a plastic bag. The plastic bag was sealed and clearly labelled with details of the animal number, location, and sex and stored at room temperature.

Table 2.1. Goat populations sampled

Province	District/ Locality	Farm	Number of samples
Gaza	Pafuri sede	A	1
Gaza	Pafuri/ Chicumba	B	5
Gaza	Pafuri/ Chicumba	C	6
Gaza	Pafuri/ Chicumba	D	1
Gaza	Pafuri/ Chicumba	E	4
Gaza	Pafuri/ Mbuzi	F	5
Gaza	Pafuri/ Mbuzi	G	6
Gaza	Pafuri/ unknown	H	5
Gaza	Pafuri/ Mungaban	I	3
Gaza	Pafuri/Salane	J	4
Tete	Changara	Cachembe	4
Tete	Marara/ PFP	PFP	11
Tete	Marara/ Centro	K	5
Tete	Marara/ Centro	L	4
Tete	Marara/ P8	M	6
Tete	Marara/ P8	N	2
Tete	Matambo	O	8
Maputo	Magude	Chemane	6
Maputo	Magude	P	6
Maputo	Chobela	Zootecnic estation	12
Maputo	Chobela	R	5
Maputo	Impauto	Reproduction center	11
Cabo Delgado	Miesi	AA	6
Cabo Delgado	Miesi	AB	5
Cabo Delgado	Pemba Metuge	Nancaramo	5
Cabo Delgado	Pemba Metuge	Nalia	4
Cabo Delgado	Pemba Metuge	Naminete	2
Cabo Delgado	Pemba Metuge	Nanduli	1
Cabo Delgado	Pemba Metuge/sede	AC	6
Cabo Delgado	Mecufi	AD	2
Cabo Delgado	Mecufi/ Murrebue	AE	5
Cabo Delgado	Mecufi/ Murrebue	AF	4

2.2. DNA extraction

Approximately 8+ hair roots of each animal were cut and placed in an Eppendorf tube. DNA was extracted from the hair roots using a modified Proteinase K digestion method (Higuchi *et al.*, 1988). The extracted DNA samples were stored at -20ºC until further use.

2.3. Microsatellite markers, PCR conditions and genotyping

A total of 17 microsatellites was selected based on the degree of polymorphism and genome coverage. The selected microsatellites are indicated in Table 2.2. These microsatellite markers adhere to the standards of the International Society for Animal Genetics and the FAO. The microsatellies were multiplexed according to the dye label and product size ranges (Table 2.2). PCR reactions, PCR, PCR preparation and the PCR programme are explained in Appendix 1.

Table 2.2. Plexes, chromosome position, fluorescent label, size ranges and sequence information of microsatellites used

Microsatellite Marker	Chromosome Number	Flouorescent Label	Product Size Range	Sequence (F- forward and R- reverse)
PLEX 1				
SRCRSP24	*Unkown*	*Fam*	162-174	F 5' –AGC AAG AAG TGT CCA CTG ACA G- 3' R 5' –TCT AGG TCC ATC TGT GTT ATT GC- 3'
SRCRSP5	*21*	*Joe*	166-180	F 5' –GGA CTC TAC CAA CTG AGC TAC AAG- 3' R 5' –TGA AAT GAA GCT AAA GCA ATG C- 3'
SRCRSP8	*Unkown*	*Joe*	210-260	F 5' –TGC GGT CTG GTT CTG ATT TCA C- 3' R 5' –CCT GCA TGA GAA AGT CGA TGC TTA G- 3'
PLEX 2				
MCM527	*5*	*Tamra*	155-173	F 5' –GTC CAT TGC CTC AAA TCA ATT C- 3' R 5' –AAA CCA CTT GAC TAC TCC CCA A – 3'
INRA23	*3*	*Joe*	208-214	F 5' –GAG TAG AGC TAC AAG ATA AAC TTC- 3' R 5' –TAA CTA CAG GGT GTT AGA TGA ACT CA- 3'
BM1329	*6 (sheep)*	*Joe*	168-182	F 5' –T TG TTT AGG CAA GTC CAA AGT C- 3' R 5' –AAC ACC GCA GCT TCA TCC- 3'
OARFCB20	*2*	*Joe*	99-125	F 5' –AAA TGT GTT TAA GAT TCC ATA CAG TG- 3' R 5' –GGA AAA CCC CCA TAT ATA CCT ATA C- 3'
CSRD247	*14*	*Fam*	236-244	F 5' –GGA CTT GCC AGA ACT CTG CAA T- 3' R 5' –CAC TGT GGT TTG TAT TCA GG- 3'
ILST087	*28*	*Fam*	145-165	F 5' –AGC AGA CAT GAT GAC TCA GC- 3' R 5' –CTG CCT CTT TTC TTG AGA GC- 3'
SRCRSP23	*Unknown*	*Fam*	83-111	F 5' –TGA ACG GGT AAA GAT GTG- 3' R 5' –TGT TTT TAA TGG CTG AGT AG- 3'
PLEX 3				
OARFCB11	*2*	*Tamra*	142-150	F 5' –GGC CTG AAC TCA CAA GTT GAT ATA TCT ATC AC- 3' R 5' –GCA AGC AGG TTC TTT ACC ACT AGC ACC- 3'
ILST002	*Unkown*	*Tamra*	118-127	F 5' –TCT ATA CAC ATG TGC TGT GC- 3' R 5' –CTT AGG GGT GAA GTG ACA CG- 3'
RM004	*15*	*Joe*	138-146	F 5' –CAG CAA AAT ATC AGC AAA CCT- 3' R 5' –CCA CCT GGG AAG GCC TTT A- 3'
INRA63	*18*	*Fam*	174-190	F 5' –ATT TGC ACA AGC TAA ATC TAA CC- 3' R 5' –AAA CCA CAG AAA TGC TTG GAA G- 3'
PLEX 4				
INRA006	*3*	*Tamra*	109-123	F 5' –AGG AAT ATC TGT ATC AAC CTC AGT C- 3' R 5' –CTG AGC TGG GGT GGG AGC TAT AAA TA- 3'
MAF65	*15*	*Fam*	117-127	F 5' –AAA GGC CAG AGT ATG CAA TTA GGA G- 3' R 5' –CCA CTC CTC TGA GAA TAT AAC ATG- 3'
BM1258	*23*	*Joe*	101-105	F 5' –GTA TGT ATT TTT CCC ACC CTG C- 3' R 5' –GAG TCA GAC ATG ACT GAG CCT G(AC)- 3'

The microsatellite preparation is described in Appendix 2. The DNA amplification was performed as follows: approximately 100ng of genomic DNA was used as a template for 7µL PCR reactions and this was amplified in a Perkin Elmer Thermal Cycler 9700. Each reaction contained 0.25 units of Super Therm Gold Taq, 250µM dNTP's, to a final concentration of 25mM Tris-MgCl buffer and different concentrations of primers, the forward primer being labelled with a fluorescent dye (Appendix 2).

PCR amplicons were visualized using polyacrylamide gel electrophoresis on an ABI 377 automated sequencer. A 5% polyacrylamide gel was prepared by adding 18g of Urea, 5ml of 50% Acrylamide, 10ml of 5x TBE buffer to 25 ml of distilled water. The solution was filtered and sonified for five minutes in a sonification bath. 250µl of Ammonium Persulphate and 36µl of TEMED was added to polymerize the gel. The gel was poured and polymerized for two hours.

Samples were prepared by diluting the PCR amplicon with distilled water and adding the diluted sample to Formamide, loading buffer and a fixed size standard (in this case GeneScan ROX 350). Samples were denatured at 95°C for three minutes and immediately put on ice. 1.5µl of the respective samples was loaded into each lane. The gel was run at 51°C for two hours.

Automated analysis

The data was captured using Genescan 3.1 software and data analysis was carried out using Genotyper 2.0 to determine the fragment sizes in base pairs. The software allocated the correct allele sizes to each individual microsatellite. From this data, an allelic table was created, then stored electronically and transferred to a database. The data was converted into the applicable input file formats for statistical analyses.

2.4. Data Analysis

Allele frequencies, heterozygosity values, genetic variation estimates, phylogenetic trees and Hardy-Weinberg equilibrium were calculated using the POPGENE version1.31 (Yeh, 1999) computer programme. The RST-CALC (Goodman, 1997) programme was used to determine the genetic differentiation between the populations. The Arlequin Version 3.0 (Scheider *et al.*, 1995) was used to calculate Fst and Rst values. AMOVA was used to determine the molecular variance and the GENECLASS 2.0 (Cornuet *et al.*, 1999) programme was used for the assignment of individuals to populations. Arlequin Vers. 3.0 computer programme was used to determine genetic differentiation measures.

CHAPTER 3

RESULTS

3.1. Populations sampled

It was possible to clearly identify and distinguish between the four populations using phenotypic characteristics. These characteristics are well described for the Mozambican goat populations. The sampling process included verbal communication with the different farmers to confirm the purity as well as the representation of the samples within the population in each province.

3.2. DNA extraction

It was possible to extract quality DNA from all samples using the modified extraction technique and only eight hair roots.

3.3. Microsatellite markers, PCR conditions and genotyping

The selected microsatellite markers showed sufficient polymorphism and were well representative of covering the genome. The multiplexing of the markers contributed to a more cost effective way of handling a vast number of samples. The PCR conditions at the ARC Animal Production Institute's Animal Genetics Laboratory where the practical part of this study was conducted were well optimized as this laboratory renders a service to the livestock industry and is well equipped. This laboratory is also ISAG standardized which implies that all equipment, for example the ABI sequencer, is regularly calibrated.

3.4. Hardy Weinberg Equilibrium (HWE)

Four Mozambican goat populations comprising 160 individual samples were genotyped using 17 microsatellite markers. Using the POPGENE computer programme, the following markers were found to be in HWE ($P \leq 0.05$) disequilibrium for a specific population (Table 3.1). For the Maputo population the markers were **SRCRSP 24,**

SRCRSP 5, MCM 527, ILSTS 002, RM 004 and BM 1258 where the P value ranged from 0.00 to 0.01. The markers not in HWE in the Cabo Delgado population were **SRCRSP 24, SRCRSP 5, SRCRSP 23** with the P value ranging from 0.00 to 0.02. In the Pafuri population **OARFCB 20** and **ILSTS 002** with P values of 0.00 and 0.04 respectively were not in HWE. Three markers were found not in HWE in the Tete population, namely. **MCM 527, SRCRSP 23** and **INRA 006** with P values ranging from 0.00 to 0.004.

Table 3.1 - Hardy Weinberg Equilibrium values for four Mozambican goat populations

Microsatellite Markers	**Mozambican goat populations**			
	Pafuri	Tete	Maputo	Cabo Delgado
SRCRSP 24	0.548	0.925	0.021*	0.000***
SRCRSP 5	0.0481	0.917	0.026*	0.000***
SRCRSP 8	0.903	0.717	0.231	0.478
MCM 527	0.112	0.000***	0.011*	0.588
INRA 23	0.887	1.000	0.887	0.999
BM 1329	0.670	0.832	0.998	0.186
OARFCB 20	0.000***	0.476	0.798	0.556
CSRD 247	0.592	0.312	0.145	0.659
ILSTS 87	0.037*	0.602	0.788	0.312
SRCRSP 23	0.914	0.000***	0.203	0.019*
OARFCB 11	0.221	0.238	0.587	0.760
ILSTS 002	0.737	0.193	0.003**	0.694
RM 004	0.780	0.238	0.040*	0.204
INRA 63	0.121	0.206	0.612	0.857
INRA 006	0.111	0.039*	0.217	0.478
MAF 65	0.456	0.188	0.151	0.775
BM 1258	0.984	0.704	0.004**	0.754

*= $P < 0.05$; **= $P < 0.01$; ***$P < 0.001$

3.5. Genetic variability measures

Allele frequencies

Allele frequencies were calculated using the POPGENE statistical computer programme and are summarized in Appendix 3. Allele frequencies ranged from 0.010 to 0.99 for any specific microsatellite. Table 3.2 indicates the number of alleles as observed in each marker within each population.

Table 3.2. Number of alleles observed in each marker within the four Mozambican goat populations

Marker	**Pafuri**	**Tete**	**Maputo**	**Cabo Delgado**
SRCRSP 24	7	6	7	7
SRCRSP 5	4	5	7	7
SRCRSP 8	7	6	7	5
MCM 527	8	7	8	10
INRA 23	6	2	3	5
BM 1329	10	9	8	8
OARFCB 20	7	6	7	7
CSRD 247	5	6	5	5
ILSTS 87	9	3	3	3
SRCRSP 23	7	5	5	6
OARFCB 11	3	3	4	3
ILSTS 002	6	5	5	4
RM 004	2	3	5	2
INRA 63	5	4	3	5
INRA 006	10	8	11	6
MAF 65	8	8	9	8
BM 1258	14	9	9	10

Alleles unique to certain populations were observed. For the Pafuri population, a total of sixteen alleles was observed only in this population with a frequency ranging from 0.013 to 0.307 (SRCRSP8 1 allele; INRA23 1 allele; ILSTS87 6 alleles; SRCRSP23 1 allele; ILSTS002 1 allele; BM1329 2 alleles; BM1258 4 alleles). Nine unique alleles were observed in the Maputo population (SRCRSP8, BM1329, CSRD247, RM004 3 alleles, INRA006, BM1258; MAF65 with an allele frequency ranging from 0.01 to 0.1. The Tete population showed four unique alleles in each the following markers, namely SRCRSP8, CSRD247, OARFCB11, RM004 with a frequency of 0.01. The Cabo Delgado population showed the lowest number of unique alleles with only three identified in this population (SRCRSP8, MCM527, OARFCB20) with an allele frequency ranging from 0.01 to 0.02. These unique markers can be used to distinguish between the four Mozambican goat populations as some occur in relatively high frequencies.

The MNA ranged from 5.59 in the Tete population to 6.94 in the Pafuri population within all individuals (Table 3.3).

Table 3.3. The mean number of alleles (MNA), sample size and the observed (Ho) and expected heterozygosity (He) values for four Mozambican goat populations (Nei, 1978)

Population	MNA	Sample size	Ho	He
Pafuri	6.941	40	0.593	0.676
Tete	5.588	40	0.550	0.600
Maputo	6.235	40	0.534	0.621
Cabo Delgado	5.882	40	0.554	0.584

Heterozygosity

Heterozygosity values were calculated using the POPGENE computer programme to determine the level of genetic variation within all the populations (Table 3.3). The observed heterozygosity (Ho) values ranged from 53% for the Maputo population to 59% for the Pafuri population. The average observed heterozygosity estimate for all populations was 56%. The lowest expected heterozygosity (He) was observed in the

Cabo Delgado population (58%) which was lower than the average of 62%. The Pafuri goat population is the most diverse goat population within Mozambique with a value of 68%.

Genetic distances

The allele frequencies were used to determine the genetic distances between the different populations. The genetic distance estimates of Nei, 1972 were used and ranged from 0.037 to 0.205. The smallest genetic distance was observed between the Maputo and Tete populations with the biggest distance between the Maputo and Pafuri populations. There was little difference in the genetic distance between the Pafuri on the one hand and the Cabo Delgado and Maputo populations on the other (Table 3.4).

Table 3.4. Genetic distance estimates of Mozambican goat populations according to Nei (1972)

	Pafuri	**Tete**	**Maputo**
Tete	0.1727	-	
Maputo	0.2054	0.0371	-
Cabo Delgado	0.2051	0.1143	0.1064

Phylogenetic analysis

The genetic distance estimates were used to construct the phylogenetic tree using the POPGENE computer programme. The phylogenetic tree supports the genetic distance estimates where the Pafuri population is the most genetically distant from the Cabo Delgado population. The Tete and the Maputo populations formed a separate cluster indicating a close relationship between these two populations.

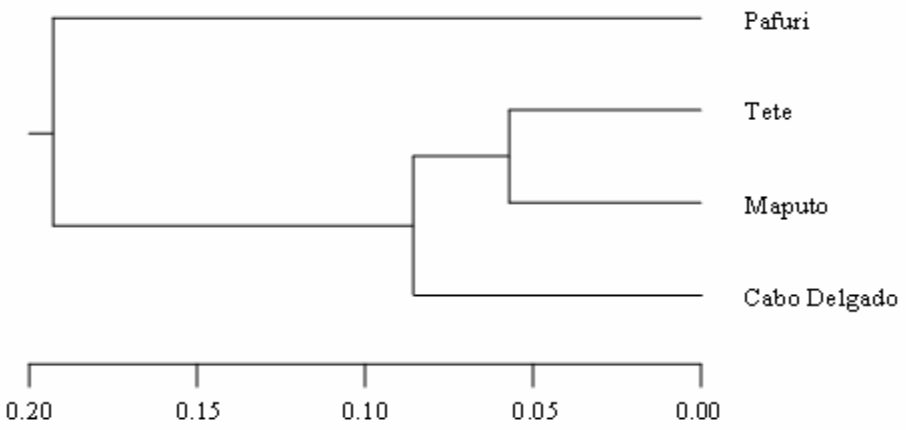

Figure 3.1. Dendrogram of genetic distance between four Mozambican goat populations revealed by UPGMA based on Nei (1978)

Genetic differentiation

The gene flow between the different populations was determined pair-wise using the POPGENE computer programme. The different values are indicated in the top diagonal in Table 3.5. The highest gene flow (8.36) was observed between the Tete and Maputo populations and the lowest (4.31) between the Pafuri and Cabo Delgado populations. The genetic differentiation measures were determined with the Arlequin Vers. 3.0 computer programme.

Table 3.5. Gene flow (above diagonal) and Fst estimates (below diagonal) determined pair-wise between four Mozambican goat populations.

	Pafuri	**Tete**	**Maputo**	**Cabo Delgado**
Pafuri	-	5.257	4.759	4.314
Tete	0.055	-	8.361	6.495
Maputo	0.073	0.047	-	7.204
Cabo Delgado	0.081	0.059	0.060	-

The Fst values range from 0.047 (Tete and Maputo) to the highest genetically different populations with a value of 0.081 (Pafuri and Cabo Delgado) (Table 3.5). The results

indicate that the Pafuri and Cabo Delgado populations are the most distinct within all the Mozambican goat populations.

Another analysis was performed using the R_{ST} calc computer programme to determine R_{ST} values between the populations in a pair-wise fashion. The values are indicated in Table 3.6. Similar results were obtained for the Fst and gene flow estimates using the Arlequin and POPGENE computer programmes between the Pafuri and Cabo Delgado populations. However, unexpected and different values were observed for R_{ST} and gene flow where the Cabo Delgado and Tete populations showed the lowest genetic differentiation. When compared to the Fst and gene flow values using the Arlequin and POPGENE computer programmes, the least differentiation was found between the Tete and Maputo populations.

Table 3.6. Matrix of R_{ST} values (below the diagonal) and gene flow values (above diagonal) to p value of 0.001

	Pafuri	**Tete**	**Maputo**	**Cabo Delgado**
Pafuri	-	1.4748	1.2564	1.2493
Tete	0.1449	-	5.1362	5.5254
Maputo	0.1660	0.0464	-	3.1522
Cabo Delgado	0.1667	0.0433	0.0735	-

Analysis of molecular variance (AMOVA)

In order to understand partitioning of the level of genetic diversity of Mozambican goats, an AMOVA analysis was conducted. The results revealed that 93.72% of the total genetic diversity occurred within the populations while 6.28% occurred amongst the populations.

Assignment test (Geneclass 2.)

A total of 135 goats (84.38%) were correctly assigned within each of the four populations. The confidence level was 99% (Appendix 3). The highest number of miss-

assigned animals was found within the Maputo population with a total of nine goats (22.5%) where five of them are supposed to belong to the Tete population according to the assignment test. Within the Pafuri population a total of seven goats (17.5%) were missed classified. The majority of the Pafuri miss-assigned animals (4) belong to the Maputo goat population according to the assignment test. Within the Cabo Delgado population, six goats (15%) were missed assigned and the highest number of animals miss-assigned was four assigned as the Tete population. Four goats (10%) within the Tete population were miss-assigned with 2 supposed to belong to the Maputo goat population according to the assignment test.

CHAPTER 4

DISCUSSION

This study is the first contribution towards the genetic characterization of the Mozambican goat populations. A total of four goat populations were identified within each of the four provinces of Mozambique according to phenotypic descriptions. These populations were identified as part of a survey conducted for the State of the World Report of the FAO, report of Commission on Genetic Resources (2004). Through this report the importance of using molecular markers for the genetic description of breeds was highlighted. Thus a genetic characterization study was initiated to contribute to the genetic characterization of different breeds, ecotypes or different populations to be conserved in Mozambique.

The study was performed at the ARC- Animal Genetics Laboratory where the expertise and equipment were available. A total of 160 animals were genotyped. These animals were representative of the four different populations or breeds as identified in the phenotypic survey. In the genetic study a total of 17 microsatellites was used to determine HWE, allele frequencies, mean number of alleles, heterozygosity, genetic distance, genetic differentiation, analysis of molecular variance (AMOVA) and assignment test.

HWE

The present study had fewer loci not in Hardy-Weinberg equilibrium compared with other studies (Thilagam, 2006; Kim *et al*, 2002; Li *et al*, 2002; Barker, 2001) that had only four in twenty loci in Hardy-Weinberg equilibrium. However, as in the Kannidu goats of Tamilnadu, India, (Thilagam, 2006), in the present study of genetic characterization using microsatellite markers, ILSTS002 was found in HWE disequilibrium for the Maputo and Pafuri goat populations. The same occurred with OARFCB20 which was found to be not in HWE in the Pafuri population. In the Li *et al* (2002), study it was not in HWE in five of 13 populations.

Genetic variability

Genetic variation is based on DNA information and it is a tool for the conservation of genetic resources. It provides information on the relationships and variation within and between populations or breeds that may be used to determine in what way the population should be conserved as a genetic resource.

Allele frequencies

All the microsatellite markers tested were found to be polymorphic in all populations. For the 17 markers tested in this study, the number of alleles observed ranged from two to 14 (Table 3.1). In studies of genetic distance, Li *et al.,* (2002) and Yang *et al.,* ((1999) suggested that microsatellite loci should have no less than four alleles per locus to reduce the standard error of distance estimates but, in the Saitbekova *et al.,* (1999) study, the observed number of alleles ranged from 2 to 19. The number of alleles observed in this study was compared to previous studies with similar microsatellite markers (Saitbekova *et al*., 1999, Yang *et al*., 1999, Luikart *et al.*, 1999, Barker *et al*., 2001, Li e*t al.,* 2002, Martínez *et al*., 2004, Thilagam *et al*., 2006). The average tendency was observed to be similar with some exceptions. The INRA 006 marker in the present study showed 10 and 11 alleles in the Pafuri and Maputo populations respectively, while the highest number found in previous studies was eight (Martínez *et al*., 2004). The BM 1329 marker showed ten alleles against nine in the same marker reported by Martínez *et al.,* (2004).

Of the 17 markers 14 **(**SRCRSP 8, MCM 527, INRA 23, ORFCB 20, CSRD 247, ILSTS 87, ILSTS 002, SRCRSP 23, OARFCB 11, RM 004, INRA 006, BM 1258, BM 1329, MAF 65), which correspond to 82.35%, were observed with at least one unique allele in individual goat populations (Appendix 3). The OARFCB 11 and ORFCB 20 markers were found to present unique alleles in individual breeds in the determination of genetic relationship among five indigenous goat breeds with six microsatellite markers (Yang *et al.,* 1999). As in the present study, the ORFCB 20 marker showed a great frequency of unique alleles. MCM 527 was found to be a unique allele present in a single population

by Thairu-Muigai (2002). This means that those markers that present unique alleles can be useful to identify specific populations or breeds.

MNA and Heterozygosity

The mean number of alleles and expected heterozygosities are good indicators of genetic polymorphism within breeds. When compared to the Kalahari Red goat breed from South Africa (Kotzé *et al.*, 2004), the Mozambican goats showed the lowest mean number of alleles ranging from 5.58 in Tete goats to 6.94 in the Pafuri population against 7.77. Generally the mean number of alleles is highly dependent on the sample size because of the unique alleles in populations, which occur in low frequencies and also because the number of observed alleles tends to increase depending on the population size. All individuals in the population were considered (Table 3.3). The expected heterozygosities (He) values per population were similar, ranging from 0.58 in the Cabo Delgado population to 0.68 in the Pafuri population (Table 3.3). Similar He values using microsatellite markers in diversity studies in goats were reported (Saitbekova *et al.,* 1999; Barker *et al.*, 2001; Kotzé *et al.,* 2004; Visser *et al.*, 2004) and these were lower than those reported by Martínez *et al,* (2004). The average observed heterozygosity was less than expected for all populations and this could be due to any one or more of the following: segregation of nonamplifying (null) alleles, scoring bias (heterozygotes scored incorrectly), selection against heterozygotes or inbreeding . Similar results were reported by Barker *et al.*, (2001) on indigenous south-east Asian goat populations.

Genetic distance

The genetic distance calculated by (Nei 1972) showed that the smallest genetic distance was between the Maputo and Tete goats with a genetic distance of 0.085 (Table 3.4). The Tete Province has the majority number of goat population of Mozambique. In the other end there is a large influx of Tete goats to the Maputo Province to restock the numbers lost during the war and to be sold for meat. This can possibly explain these result of the small genetic distance, because of the mixing of these two populations although the

samples were taken in places were the owners declared that there was no mixing of Tete and Maputo goats in their herds.

The largest genetic distance was observed between the Maputo and Pafuri populations and the Pafuri and Cabo Delgado populations. This can be due to the fact that they have been described to have different origins. The Maputo, Tete, and Cabo Delgado goats belong to the same breed and are described phenotypically as Landim goats (Gall, 1996; Morgado, 2000). On the other hand, Pafuri goats are found in a specific arid area of Mozambique called the Pafuri District and they resulted from a crossbreeding between Boer males and Landim females (Gall, 1996).

In studies conducted in South Africa on genetic distances between different goat breeds, it was found that this panel of 17 microsatellites was sufficient to determine the difference between breeds (Visser *et al*, 2004, Kotzé *et al*, 2004). The reliability of these markers can thus contribute to the accurate identification of breeds.

Genetic differentiation

The highest gene flow (8.36) was observed between the Tete and Maputo populations and the lowest (4.31) between the Pafuri and Cabo Delgado populations (top diagonal in Table 3.5). The Fst values ranged from 0.047 (Tete and Maputo) to the highest genetically different populations with a value of 0.081 (Pafuri and Cabo Delgado) (below diagonal Table 3.5).

The results indicate that the Pafuri and Cabo Delgado populations are the most distinct within all the Mozambican goat populations. That can be explained by the distance between them as can seen on the map of sample collection (Figure 3.1) and the different breeds to which they belong (Gal, 1996). The genetic differentiation observed among populations was high ($P< 0.001$) for all population even where a low Fst value obtained, indicating that there was a significant level of differentiation between the populations. That difference between the populations may suggest local selection and the presence of possible ecotypes or breeds in distinct regions and environment.

Analysis of molecular variance (AMOVA)

In order to understand the partitioning of the level of genetic diversity of Mozambican goats, an AMOVA analysis was conducted. The results revealed that 93.72% of the total genetic diversity occurred within the populations while 6.28% occurred amongst the populations. The values were lower than those found in the genetic diversity in Swiss goat breeds based on microsatellite markers by Saitbekova *et al* (1999), who found that 17% of the total diversity was between breeds and 83% of the diversity was within breeds. In the genetic relationships among 12 Chinese indigenous goat populations based on microsatellite analysis by Li *et al* (20029, 10.5% of the total variation was between populations and 89.5% was within populations. This was also found by Chenyambuga (2002), in the genetic characterization of indigenous goat populations of sub-Saharan Africa using microsatellite DNA markers.

Assignment test (Geneclass 2.)

A total of 135 goats (84.38%) were correctly assigned within each of the four populations. The confidence level was 99% (Appendix 3). Within the Maputo population a total of (22.5%) goats were missed and the highest number of miss-assigned animals was five missed in the Tete population. Within the Pafuri population a total of (17.5%) goats were missed and the highest number of miss-assigned animals was found as the Maputo goat population (4). In the Cabo Delgado population six goats (15%) were missed and the highest number of animals miss-assigned was four assigned within the Tete population. A total of (10%) goats within the Tete population were miss-assigned.

In general, the results showed that the highest miss-assignment was found in the Maputo goat populations that were miss assigned within the Tete goat population. In concordance with genetic differentiation, the highest gene flow (8.36) was observed between the Tete and Maputo populations and this can be supported by their origin (phenotypic classification) and the influx of goats from Tete to Pafuri. Unexpected results were shown in the Pafuri with four goats miss-assigned within the Maputo and two within the Tete populations. The possible reason is that these breeds are crossbreeds between the Landim

goat where the Maputo and Tete goats belong and the Boer breed (Gall, 1996; Morgado, 2000). The assignment of the Cado Delgado goat population is in concordance with the analysis performed using the R_{ST}-calc computer programme to determine R_{ST} values between the populations in a pairwise fashion (Table 3.6), where values observed for R_{ST} and gene flow showed the lowest genetic differentiation between the Cabo Delgado and Tete populations.

CHAPTER 5

CONCLUSION

This is the first time that the Mozambican goats have been studied at molecular level. This study provides important information for the future conservation of Mozambican goat resources**.** Therefore, it is a powerful tool for breeding improvement because it will allow the preservation of the local breeds and control of crossbreeding in future restocking programs.

The microsatellite markers used in this study were found to be useful and informative for studying the genetic diversity in Mozambican goats.

The genetic diversity of the Mozambican goat population was high, as indicated by the mean number of alleles and expected heterozygosities observed for the populations. The results of the AMOVA showed that most of the diversity for the Mozambican goat populations is found within populations, rather than between any geographical or phenotypic groupings.

The genetic distance results revealed a closer relationship between the Tete and Maputo goat populations.

There is sufficient genetic variation within Mozambican goat populations, with distinct genetic differentiation between the Cabo Delgado and Pafuri goats and the Maputo and Pafuri goats that can suggest that they are really different breeds.

In the assignment test, 84.38% were correctly assigned to their original population. So, the four study populations are genetically different.

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APPENDIX

APPENDIX 1

1. PCR Reagents

1. 100pmol/µl Reverse and Forward Microsatellite primers:
2. Supertherm Gold DNA polymerase 5U/µl (Cat. # JMR851)
3. 10 x Reaction Buffer containing 15mM $MgCl_2$ (Cat. # JMR470)
4. dNTP's 2.5mM each (TaKaRa Biochemicals Cat # 4030)

2. PCR

PLEX 1

PCR	**1 Reaction**
Primer	0.9
Taq	0.4
dNTP	1.6
Buffer	1.5
Water	2.8
DNA	1
Total	**8.2**

PLEX 2

PCR	**1 Reaction**
Primer	2.1
Taq	0.4
dNTP	1.6
Buffer	1.5
Water	1.6
DNA	1
Total	**8.2**

PLEX 3

PCR	**1 Reaction**
Primer	1.2
Taq	0.4
dNTP	1.6
Buffer	1.5
Water	2.5
DNA	1
Total	**8.2**

PLEX 4

PCR	**1 Reaction**
Primer	1.5

Taq	0.4
dNTP	1.6
Buffer	1.5
Water	2.2
DNA	1
Total	**8.2**

3. PCR Preparation:

0.3 µl/ Primer (F+R)
5 Units/µl (Supertherm Gold)
2.5 mM of each dNTP (Takara)
10 x reaction Buffer (15mM MgCl2, 100mM Tris (hydroxymethyl aminomethane Hydrochloride (Tris-HCl), pH 8.3 at 25°C, 500mM KCl - Takara

MgCl2	15mM
Tris-HCl	100mM
KCl	500mM

Final concentration in each tube:

Buffer

MgCl2	2.445mM
Tris-HCl pH	16.304mM
KCl	81.521mM
dNTPs	434.7826 µM each
Primers	0.04 – 0.9 µM each F/R primer (typical)

Final quantity in each tube:

Taq	2 units
DNA	40 ng template DNA
F-primers (mean)	2.607727 pmoles each
R-primers (mean)	2.607727 pmoles each
Mean primer quant per locus	5.215454 pmoles each

Final tube quantity / µl:

Taq	0.217 units/µl
DNA	4.347826 ng/µl
F-Primer (mean)	0.283448 pmoles/µl for each forward primer
R-Primer (mean)	0.283448 pmoles/µl for each reverse primer
Mean Primer Quantity per locus	0.566897 pmoles/µl for each F+R primer set
dNTPs	0.4347826 nMoles/µl each

4. PCR Programme:

12 minutes 94°C Hot-Start
33 cycles (94°C for 45 sec; 60°C for 80 sec; 72°C for 60 sec)
72°C for 60 min
4°C for ∞

APPENDIX 2

Microsatellite preparation

Mix the 100pmol/µl microsatellite oligonucleotides in one 1.5 ml Eppendorf tube according to the following tabulation:

PLEX 1

Primer	Volume	Optimum Conc(pMol/µl from dilution series	pMoles per tube	Final Conc (µM)
SRCRSP24	0.3	28	3.5	0.380
SRCRSP5	0.3	28	3.5	0.380
SRCRSP8	0.3	28	3.5	0.380
TOTAL	**0.9**	**84**	**10.5**	**1.14**

PLEX 2

Primer	Volume	Optimum Conc(pMol/µl from dilution series	pMoles per tube	Final Conc (µM)
MCM527	0.3	18	2.25	0.245
INRA23	0.3	20	2.5	0.271
BM1329	0.3	12	1.5	0.163
OARFCB20	0.3	6	0.75	0.081
CSRD247	0.3	6	0.75	0.081
ILSTS087	0.3	10	1.25	0.136
SRCRSP23	0.3	14	1.75	0.190
TOTAL	**2.1**	**86**	**10.25**	**2.167**

PLEX 3

Primer	Volume	Optimum Conc(pMol/µl from dilution series	pMoles per tube	Final Conc (µM)
OARFCB11	0.3	48	6	0.652
ILSTS002	0.3	36	4.5	0.489
RM004	0.3	8	1	0.108
INRA63	0.3	3	0.375	0.040
TOTAL	**1.2**	**9.5**	**11.87**	**1.289**

PLEX 4

Primer	Volume	Optimum Conc(pMol/ µl from dilution series	pMoles per tube	Final Conc (µM)
INRA006	0.3	54	6.75	0.733
BM1818	0.3	60	7.5	0.815
MAF65	0.3	10	1.5	0.163
CSSM36	0.3	24	3	0.362
BM1258	0.3	48	6	0.652
TOTAL	**1.5**	**196**	**24.75**	**2.725**

APPENDIX 3.

Allele frequencies across all population and total number of alleles per locus and per populations

SRCRSP 24	Pafuri	Tete	Maputo	Cabo Delgado
A	0.500	0.725	0.338	0.350
B				
C	0.100	0.038	0.013	
D			0.188	0.063
E	0.138	0.125	0.225	0.300
F	0.038	0.013		0.038
G	0.175	0.038	0.100	
H			0.113	
I				
J	0.025	0.063		0.088
K				
L			0.025	0.113
M	0.025			0.050
N				
O				
P				
Q				
Alleles number	7	6	7	7

SRCRSP 5	Pafuri	Tete	Maputo	Cabo Delgado
A	0.385	0.300	0.188	0.338
B	0.013	0.013	0.050	0.100
C			0.100	0.188
D	0.474	0.438	0.238	0.188
E			0.013	
F	0.128	0.137	0.075	0.013
G		0.113	0.338	0.175
H				
I				
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	4	5	7	7

SRCRSP8	Pafuri	Tete	Maputo	Cabo Delgado
A		*0.013		
B	0.013	0.025	0.013	
C				
D	0.026	0.038	0.025	
E		0.05		
F				
G				*0.013
H	0.692	0.525	0.663	0.675
I				
J				
K	0.051		0.075	0.038
L				
M	0.141	0.35	0.063	0.25
N	0.064		0.125	0.025
O	*0.013			
P				
Q			*0.038	
Alleles number	7	6	7	5

MCM 527	Pafuri	Tete	Maputo	Cabo Delgado
A				
B				*0.013
C	0.013		0.075	0.038
D		0.013		0.013
E				
F	0.113	0.338	0.163	0.15
G				
H				
I				
J	0.213	0.35	0.213	0.275
K	0.325	0.05	0.013	0.025
L	0.013	0.038	0.025	
M	0.175	0.025	0.063	0.025
N	0.137	0.188	0.363	0.375
O	0.013			0.025
P			0.088	0.063
Q				
Alleles number	8	7	8	10

INRA 23	Pafuri	Tete	Maputo	Cabo Delgado
A	0.713	0.988	0.975	0.9
B				
C	0.025		0.013	0.025
D	0.013	0.013		0.013
E				
F	0.025			0.038
G				
H	0.088		0.013	0.025
I	*0.138			
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	6	2	3	5

BM 1329	Pafuri	Tete	Maputo	Cabo Delgado
A	0.025		0.038	
B	0.45	0.013		
C	0.1	0.038		0.075
D	0.012	0.038		
E	0.15	0.35	0.55	0.288
F	*0.025			
G	*0.063			
H	0.113	0.038	0.025	0.162
I		0.025	0.013	0.113
J	0.025	0.2	0.275	0.15
K		0.125		0.025
L			*0.013	
M	0.038	0.175	0.013	0.113
N			0.075	0.075
O				
P				
Q				
Alleles number	10	9	8	8

OARFCB 20	Pafuri	Tete	Maputo	Cabo Delgado
A				
B	0.167	0.275	0.313	0.138
C	0.017		0.025	0.013
D	0.267	0.2	0.238	0.45
E				
F	0.167	0.463	0.2	0.225
G				
H	0.233	0.013	0.175	0.138
I	0.117	0.037	0.038	
J	0.033	0.013	0.013	0.013
K				
L				*0.025
M				
N				
O				
P				
Q				
Alleles number	7	6	7	7

CSRD 247	Pafuri	Tete	Maputo	Cabo Delgado
A	0.075	0.038		
B	0.475	0.5	0.513	0.688
C	0.2	0.175	0.213	0.038
D	0.188	0.05	0.163	0.038
E				0.012
F	0.063	0.225	0.088	0.225
G				
H				
I			*0.025	
J		*0.013		
K				
L				
M				
N				
O				
P				
Q				
Alleles number	5	6	5	5

ILSTS 87	Pafuri	Tete	Maputo	Cabo Delgado
A	0.177	0.75	0.763	0.718
B	*0.016			
C	*0.307			
D				
E	*0.129			
F	*0.016			
G	0.032	0.238	0.213	0.244
H	0.016	0.013	0.025	0.039
I	*0.242			
J	*0.065			
K				
L				
M				
N				
O				
P				
Q				
Alleles number	9	3	3	3

SRCRSP 23	Pafuri	Tete	Maputo	Cabo Delgado
A	0.45	0.313	0.575	0.088
B	0.438	0.4	0.338	0.638
C	0.013	0.063	0.05	0.038
D	0.05	0.075		0.138
E	*0.013			
F	0.025		0.013	0.025
G	0.013	0.15	0.025	0.075
H				
I				
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	7	5	5	6

OARFCB 11	Pafuri	Tete	Maputo	Cabo Delgado
A			0.013	0.013
B		*0.013		
C	0.075		0.013	
D	0.675	0.463	0.397	0.625
E	0.25	0.525	0.577	0.363
F				
G				
H				
I				
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	3	3	4	3

ILSTS 002	Pafuri	Tete	Maputo	Cabo Delgado
A	0.038	0.025	0.05	
B	0.513	0.663	0.575	0.763
C	*0.013			
D	0.087	0.025	0.05	0.1
E	0.263	0.2	0.275	0.088
F	0.087	0.088	0.05	0.05
G				
H				
I				
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	6	5	5	4

RM 004	Pafuri	Tete	Maputo	Cabo Delgado
A			*0.051	
B	0.738	0.463	0.359	0.55
C		*0.013		
D	0.263	0.525	0.539	0.45
E			*0.013	
F			*0.039	
G				
H				
I				
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	2	3	5	2

INRA 63	Pafuri	Tete	Maputo	Cabo Delgado
A	0.388	0.513	0.463	0.65
B	0.363	0.288	0.45	0.25
C				
D	0.013	0.025		0.013
E	0.188	0.175	0.088	0.063
F				
G	0.05			0.025
H				
I				
J				
K				
L				
M				
N				
O				
P				
Q				
Alleles number	5	4	3	5

INRA 006	Pafuri	Tete	Maputo	Cabo Delgado
A	0.138		0.063	0.063
B	0.025	0.013	0.025	
C	0.087	0.05	0.05	0.013
D	0.125	0.2	0.325	0.363
E	0.113	0.075	0.038	
F	0.363	0.513	0.25	0.1
G	0.038	0.1	0.025	0.25
H	0.05	0.025	0.063	
I	0.05	0.025	0.025	0.212
J	0.013		0.125	
K			*0.013	
L				
M				
N				
O				
P				
Q				
Alleles number	10	8	11	6

MAF 65	Pafuri	Tete	Maputo	Cabo Delgado
A				
B	0.037	0.212	0.063	0.113
C				
D		0.025	0.038	0.038
E	0.362	0.425	0.525	0.675
F			0.013	0.05
G	0.012	0.05	0.013	0.013
H			*0.1	
I	0.113	0.025		0.025
J	0.037			
K	0.163	0.088	0.012	0.063
L	0.138	0.013	0.163	0.025
M				
N	0.138	0.163	0.075	
O				
P				
Q				
Alleles number	8	8	9	8

BM 1258	Pafuri	Tete	Maputo	Cabo Delgado
A	0.113	0.038	0.138	0.013
B	0.250	0.237	0.350	0.475
C	0.150	0.325	0.088	0.013
D	0.062	0.113	0.025	0.050
E	0.037	0.025	0.025	0.025
F	0.113	0.138	0.075	0.113
G	0.087	0.063	0.113	0.238
H	0.050	0.013		0.038
I	0.075	0.050	0.163	0.025
J	*0.013			
K	*0.013			
L	*0.013			
M	*0.013			
N			*0.025	
O	0.013			0.012
P				
Q				
Alleles number	14	9	9	10

Red color – least values
Blue color – Highest Values
***- Unique allele in population**

APPENDIX 4.

Assignment test

```
GeneClass 1.0.02 (16.II.99)
COMPUTATION STARTED. Date: 10/11/2006  Time: 07:32:00.
REFERENCE FILE: C:\Program Files\GeneClass\Goat assign.dat
TITLE: Title line:"Goats"
SELECTED LOCI: BM1258, BM1329,CsRd247,ILSTS002,ILStS087,INRA006,INRA023,
INRA63,MAF65,MCM527,OARFCB11,OarFcb20,RM004,SrCrsP23,SrCrSp24,SrCrSp5,
SrCrSp8
SELF CLASSIFICATION
"Frequency" Method.
"Leave One Out" procedure.
assume constant frequency=0.01 in case of null frequency.
135 individuals on 160 correctly identified (84.38%)
```

num.	name	group	loc.	P42	T82	M122	C162	classified in
1	P3	[P42]	16	18.02	20.51	21.50	24.55	=
2	P4	[P42]	17	22.09	22.86	24.09	28.01	=
3	P5	[P42]	15	13.43	15.99	17.70	19.55	=
4	P6	[P42]	16	17.10	24.92	28.45	28.85	=
5	P7	[P42]	15	19.22	19.15	18.94	24.33	M122
6	P8	[P42]	17	17.25	25.37	27.06	25.09	=
7	P9	[P42]	17	20.55	24.31	29.66	29.53	=
8	P10	[P42]	17	16.38	27.51	25.66	28.23	=
9	P11	[P42]	17	16.49	27.65	25.70	23.35	=
10	P12	[P42]	17	18.31	27.10	30.25	30.82	=
11	P13	[P42]	17	17.12	24.74	25.48	23.69	=
12	P14	[P42]	17	20.32	28.58	27.68	29.65	=
13	P15	[P42]	16	22.96	24.92	27.36	33.27	=
14	P16	[P42]	17	18.72	23.39	25.16	25.02	=
15	P17	[P42]	17	17.53	23.52	26.82	25.32	=
16	P18	[P42]	17	19.10	27.51	28.71	30.59	=
17	P19	[P42]	17	20.87	23.72	25.98	26.58	=
18	P20	[P42]	16	18.36	25.28	26.95	28.96	=
19	P21	[P42]	16	19.35	29.60	29.81	31.43	=
20	P22	[P42]	16	16.79	25.80	26.29	28.00	=
21	P23	[P42]	17	22.48	27.60	30.55	29.33	=
22	P24	[P42]	15	19.84	20.70	23.61	22.40	=
23	P25	[P42]	16	19.13	23.65	25.22	30.86	=
24	P26	[P42]	17	18.36	28.26	26.59	27.28	=
25	P27	[P42]	17	16.99	28.01	28.09	30.23	=
26	P28	[P42]	17	16.30	19.82	23.92	20.88	=
27	P29	[P42]	17	15.79	21.79	21.56	20.72	=
28	P30	[P42]	15	17.56	20.72	23.08	24.14	=
29	P31	[P42]	16	17.21	23.09	22.20	22.52	=
30	P32	[P42]	17	16.96	19.50	20.58	25.27	=
31	P33	[P42]	16	23.07	25.55	30.41	28.04	=
32	P34	[P42]	16	19.55	19.47	23.54	22.94	T82
33	P35	[P42]	17	16.37	23.92	22.73	25.68	=
34	P36	[P42]	16	17.25	20.83	22.53	26.89	=
35	P37	[P42]	15	20.29	18.19	19.38	16.83	C162
36	P38	[P42]	17	24.49	22.59	21.17	24.88	M122
37	P39	[P42]	17	22.08	19.03	19.05	19.28	T82

38	P40	[	P42]	17	21.43	26.08	20.98	28.21	M122
39	P41	[	P42]	17	19.43	21.99	21.28	22.32	=
40	P42	[	P42]	17	19.36	23.72	18.67	23.96	M122
41	T43	[	T82]	17	18.76	13.37	16.30	16.99	=
42	T44	[	T82]	17	19.01	14.30	17.09	16.58	=
43	T45	[	T82]	17	19.80	13.87	16.16	15.42	=
44	T46	[	T82]	17	18.94	12.80	16.22	15.63	=
45	T47	[	T82]	17	26.08	18.66	20.41	19.77	=
46	T48	[	T82]	17	22.58	16.23	16.60	18.69	=
47	T49	[	T82]	17	21.05	14.38	18.13	16.66	=
48	T50	[	T82]	17	19.88	15.15	16.92	15.20	=
49	T51	[	T82]	17	17.46	11.60	15.16	14.10	=
50	T52	[	T82]	17	20.78	14.70	21.34	20.84	=
51	T53	[	T82]	17	19.18	15.66	19.93	21.43	=
52	T54	[	T82]	17	19.06	13.46	15.39	17.20	=
53	T55	[	T82]	17	18.09	16.25	16.17	19.67	M122
54	T56	[	T82]	17	18.47	15.26	21.07	21.40	=
55	T57	[	T82]	17	20.11	13.95	15.63	17.63	=
56	T58	[	T82]	17	20.79	14.02	17.20	17.45	=
57	T59	[	T82]	17	20.41	13.43	16.24	16.95	=
58	T60	[	T82]	17	21.41	15.86	20.76	22.60	=
59	T61	[	T82]	17	19.51	13.79	20.05	17.00	=
60	T62	[	T82]	17	18.43	14.24	19.76	18.43	=
61	T63	[	T82]	17	21.33	19.61	16.81	21.84	M122
62	T64	[	T82]	17	18.39	14.09	17.54	18.95	=
63	T65	[	T82]	17	20.90	14.26	19.57	22.45	=
64	T66	[	T82]	17	21.27	16.80	19.18	20.68	=
65	T67	[	T82]	17	20.45	13.95	16.24	17.41	=
66	T68	[	T82]	17	22.08	15.57	17.64	17.70	=
67	T69	[	T82]	17	20.57	14.62	17.91	20.19	=
68	T70	[	T82]	17	18.91	13.47	18.33	18.45	=
69	T71	[	T82]	17	20.36	14.79	20.25	15.49	=
70	T72	[	T82]	17	19.10	19.70	20.90	23.30	P42
71	T73	[	T82]	17	20.59	14.47	18.96	18.56	=
72	T74	[	T82]	17	19.59	16.14	17.08	17.17	=
73	T75	[	T82]	17	22.35	13.63	16.80	17.83	=
74	T76	[	T82]	17	22.97	15.35	21.05	20.19	=
75	T77	[	T82]	17	25.84	19.06	23.70	25.55	=
76	T78	[	T82]	17	19.59	17.78	21.22	23.55	=
77	T79	[	T82]	17	15.21	12.20	13.31	12.82	=
78	T80	[	T82]	17	19.82	19.20	21.28	22.11	=
79	T81	[	T82]	17	21.53	16.73	17.52	26.26	=
80	T82	[	T82]	17	20.75	20.58	24.46	24.60	=
81	M83	[	M122]	17	17.83	17.21	18.77	16.06	C162
82	M84	[	M122]	17	20.81	18.65	18.29	19.91	=
83	M85	[	M122]	17	26.93	22.77	23.09	24.47	T82
84	M86	[	M122]	17	24.31	20.23	16.52	21.25	=
85	M87	[	M122]	17	17.98	17.79	14.94	15.20	=
86	M88	[	M122]	17	20.35	15.23	14.41	14.89	=
87	M89	[	M122]	17	15.91	13.33	13.57	14.31	T82
88	M90	[	M122]	17	16.53	14.26	16.84	14.59	T82
89	M91	[	M122]	17	15.79	14.33	14.72	16.90	T82
90	M92	[	M122]	17	19.93	14.42	15.08	16.25	T82
91	M93	[	M122]	17	15.43	12.46	12.23	13.46	=
92	M94	[	M122]	17	21.02	18.89	18.32	25.96	=
93	M95	[	M122]	17	21.51	20.50	16.36	24.32	=
94	M96	[	M122]	17	22.64	18.85	14.14	19.63	=

95	M97	[	M122]	17	26.74	26.80	19.74	30.42	=
96	M98	[	M122]	17	20.96	17.55	15.08	18.45	=
97	M99	[	M122]	17	21.18	20.81	15.07	25.16	=
98	M100	[	M122]	17	20.38	17.61	16.93	22.69	=
99	M101	[	M122]	17	19.37	16.70	15.18	20.07	=
100	M102	[	M122]	17	19.95	19.30	14.90	23.75	=
101	M103	[	M122]	17	24.99	21.88	19.65	26.62	=
102	M104	[	M122]	17	15.85	14.63	12.54	15.65	=
103	M105	[	M122]	17	24.72	19.57	18.63	18.10	C162
104	M106	[	M122]	17	25.75	22.95	19.17	19.40	=
105	M107	[	M122]	17	23.30	19.24	14.91	19.10	=
106	M108	[	M122]	17	21.18	16.04	11.92	13.95	=
107	M109	[	M122]	17	29.59	24.30	22.76	26.27	=
108	M110	[	M122]	17	23.75	20.51	15.01	16.59	=
109	M111	[	M122]	17	23.27	19.82	14.54	18.61	=
110	M112	[	M122]	17	23.92	22.53	15.39	19.15	=
111	M113	[	M122]	17	30.27	29.84	23.64	30.37	=
112	M114	[	M122]	16	27.96	24.61	16.28	23.94	=
113	M115	[	M122]	17	26.31	26.14	19.54	23.35	=
114	M116	[	M122]	17	28.39	23.52	16.78	24.10	=
115	M117	[	M122]	17	21.88	17.77	14.12	17.12	=
116	M118	[	M122]	17	20.98	19.66	16.36	15.71	C162
117	M119	[	M122]	17	24.81	22.28	16.29	22.53	=
118	M120	[	M122]	13	27.77	20.40	18.97	19.64	=
119	M121	[	M122]	16	30.93	29.91	25.94	29.91	=
120	M122	[	M122]	17	30.50	30.67	25.43	21.97	C162
121	C123	[	C162]	16	31.08	29.98	25.85	21.90	=
122	C124	[	C162]	17	26.00	20.59	20.51	15.80	=
123	C125	[	C162]	16	23.05	19.33	17.78	13.90	=
124	C126	[	C162]	16	23.38	19.95	18.65	13.10	=
125	C127	[	C162]	17	22.91	22.27	18.43	16.05	=
126	C128	[	C162]	17	23.86	18.30	16.16	12.71	=
127	C129	[	C162]	17	29.34	23.32	20.72	17.52	=
128	C130	[	C162]	17	28.82	23.93	23.24	17.15	=
129	C131	[	C162]	17	27.82	25.62	24.06	20.35	=
130	C132	[	C162]	17	25.76	22.43	19.29	17.65	=
131	C133	[	C162]	17	26.57	28.15	23.66	21.17	=
132	C134	[	C162]	17	23.29	21.86	15.99	13.39	=
133	C135	[	C162]	17	23.08	17.19	15.38	13.24	=
134	C136	[	C162]	17	23.25	19.35	18.98	17.04	=
135	C137	[	C162]	17	21.66	20.82	15.53	17.28	M122
136	C138	[	C162]	17	24.32	19.25	21.04	16.55	=
137	C139	[	C162]	17	21.85	19.11	22.09	16.30	=
138	C140	[	C162]	17	18.57	17.28	19.23	12.62	=
139	C141	[	C162]	17	20.94	15.43	16.03	16.31	T82
140	C142	[	C162]	17	20.08	16.63	21.57	15.83	=
141	C143	[	C162]	17	21.02	17.44	19.81	14.32	=
142	C144	[	C162]	17	19.27	14.83	15.31	16.88	T82
143	C145	[	C162]	17	21.85	22.58	19.80	17.92	=
144	C146	[	C162]	17	20.02	15.49	18.95	17.58	T82
145	C147	[	C162]	17	21.15	17.83	21.55	17.79	=
146	C148	[	C162]	17	16.29	12.62	15.09	10.87	=
147	C149	[	C162]	17	18.27	16.50	18.81	11.67	=
148	C150	[	C162]	17	20.13	14.61	17.20	14.29	=
149	C151	[	C162]	17	20.61	17.85	18.09	14.30	=
150	C152	[	C162]	17	17.90	13.55	16.94	13.21	=
151	C153	[	C162]	17	21.62	17.96	19.65	14.78	=

```
 152      C154  [     C162 ]  17| 19.28| 20.30| 19.93| 15.40|=
 153      C155  [     C162 ]  17| 20.22| 16.66| 15.97| 16.01|M122
 154      C156  [     C162 ]  17| 21.41| 19.44| 20.39| 15.48|=
 155      C157  [     C162 ]  17| 18.98| 18.94| 20.04| 16.52|=
 156      C158  [     C162 ]  17| 21.44| 18.45| 18.82| 15.36|=
 157      C159  [     C162 ]  17| 17.75| 13.80| 18.52| 15.34|T82
 158      C160  [     C162 ]  17| 22.42| 19.05| 20.53| 14.25|=
 159      C161  [     C162 ]  17| 18.18| 14.36| 16.11| 12.92|=
 160      C162  [     C162 ]  17| 18.57| 14.22| 14.62| 12.08|=
COMPUTATION TERMINATED. Date: 10/11/2006  Time: 07:32:00.
```