MOLECULAR AND POPULATION GENETIC ANALYSES OF COAT COLOUR IN NIGERIAN GOATS

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CERTIFICATION

THIS IS TO CERTIFY THAT THE THESIS

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ADEFENWA, MUFLIAT ADEJUMOKE

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DEDICATION

This work is dedicated to my late parents, Alhaji and Mrs. Adefenwa and to my husband, Mojeed Adams-Mogaji.

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ABSTRACT

This research was aimed at investigating the relationship between coat colour and some body traits in Nigerian goats, identify mutations in some mammalian coat colour genes in goats, determine the relationship between such variations and coat colour differences among Nigerian and some American goat breeds, and validate the association between identified mutations and some body traits in Nigerian goats.

Data on body weight and some linear body measurements were collected from West African Dwarf, Red Sokoto and Sahel goats from different states across Nigeria. Multivariate analysis of variance using the General Linear Model (GLM) procedure of SPSS (2010) was carried out for body weight and all the linear body measurements. Coat colour was found to significantly ($P<0.05$) affect body weight, body length and heart girth.

Three single nucleotide polymorphisms (SNPs), two missense mutations; p.E55K and p.N56K; and one silent mutation, *g.221T>C,* were identified, upon amplification and sequencing, within a 684 bp fragment spanning part of the coding region of the *MC1R* gene in Nigerian goats. Both missense mutations were present only in the WAD goat. Among American goats, five single nucleotide polymorphisms (SNPs), four missense mutations; p.S52R, p.E55K, p.K226E and p.R229P; and one silent mutation, *g.221C>T*, were identified within the same 684 bp region. The missense mutations p.S52R and p.E55K were found to be specific to the Alpine breed while p.K226E and p.R229P were found only in the Spanish breed. The missense mutation p.E55K was identified in both

populations and was found to be specific to the WAD goat (mostly black) and the Alpine breed (black and white).

A 352 bp fragment spanning part of exon 2 and the whole intron 2 of the *ASIP* gene was amplified and sequenced in Nigerian and American goats. Nine mutations; three intronic, one silent and five missense (p.S19R, p.N35K, p.L36V, p.M42L and p.L45W) mutations were identified in Nigerian goats. About 89% of the animals were found to carry the wild type allele. In the American goats, two intronic mutations, *g.293G>A* and *g.327C>A*, were identified. The results from the studies on *MC1R* and *ASIP* genes seem to support the hypothesis of epistatic interaction between *MC1R* and *ASIP*, i.e., the possibility to express the *ASIP* alleles can be obtained only when at least a copy of a putative wild type allele at the *MC1R* locus is present. The white phenotype in the Saanen and Sahel goats maybe due to the presence of the A^{wt} allele at the *ASIP* locus as described in other goat breeds with white coat colour. The *g.293G>A* and *g.327C>A* mutations found in the American breeds seem to be specific to the Alpine breed. These mutations might therefore contribute to the black colour observed in this breed.

No significant differences were found to exist between Nigerian breeds for the *g.469C>G* mutation at the *MLPH* locus. Lack of association between genotypes at the *MC1R* locus and morphological traits was also observed in this study

According to the results obtained from these studies, mutations at the *MC1R* and *ASIP* loci may determine eumelanic and phaeomelanic phenotypes. They are, however, probably not the only factors. Other genes might be involved in determining red coat colour especially.

1.0 Introduction and Background of Study

Livestock are found everywhere in poor communities across the developing world. An estimated two-thirds of households in rural communities keep some type of livestock (Randolph *et al.,* 2007). Similar information for poor urban households is scarce, but according to Randolph *et al.* (2007), a recent survey in two cities in Nigeria found that more than one-half of all urban households were keeping livestock; the highest rates were found in the most densely populated, lower-income areas. Small ruminants, goats and sheep, when compared to other livestock species, are extremely important within most farming systems (Djajanegara *et al.,* 1996). Small ruminants are relatively easy to own by resource poor farmers especially women because of their small size and early maturity that makes them suitable for meeting subsistence needs for meat and milk. Raising small ruminants is regarded as relatively easy with minimal inputs and low maintenance costs coupled with their ability to maximise available feed resources. In most cases it is basically a secondary activity that employs low levels of family resources (Nwafor, 2004). Particular advantages of small ruminants over large ruminants include higher production efficiency, easier marketability and lower risks (Soedjana *et al.*, 1988), broader adaptability to different environments, and smaller absolute feed requirements per animal (Peters, 1988). Small ruminants contribute immensely to the general economy of nations. Raising of small ruminants is an important economic activity from which food (meat, milk) and non-food commodities (manure, hides and skin, wool, etc) and cash income are derived.

Goats are renowned for their hardiness and prolificacy. Sheep are grazers and amenable to herding. On the other hand, goats are browsers and have the ability to thrive even when feed resources, except bushes and shrubs, appear to be non-existent (Devandra, 1989). Goats have higher survival rates under drought conditions. They can still provide a reasonable amount of production with every four days watering routine (Mengistu, 2007). There is an apparent consumers' preference for goat meat and mutton over available substitutes in most parts of West Africa. This preference is mainly associated to the taste of the meat, the dietary variety, as well as consumers' cultural and religious considerations (Sumberg and Cassaday, 1985). There is a growing increase in human population and if the production of livestock continues at its present rate, the increase in livestock production will ultimately lag behind the ever-growing human population. The Food and Agriculture Organization (FAO) in 1990 estimated that animal production should be increased by 4% annually to meet the demand of the human population.

The major factors that affect the productivity of goats, and small ruminants in general, in Sub-Saharan Africa are feed supply, genotype, animal management, policy and institutional constraints (Ibrahim, 1998; Peacock and Sherman, 2008). The major feed resource which is natural pasture shows remarkable seasonality in yield and quality and lacks the major nutrients that support animal growth particularly during the dry season (Ibrahim, 1998; Peacock and Sherman, 2008). There is a high demand for goat meat globally and with such a high demand, research should be tailored towards providing adequate information on suitable genotypes, improved management practices and other relevant packages (Ibrahim, 1998; Peacock and Sherman, 2008).

Goats (*Capra hircus*) are found across all agro-ecological environments and in nearly all livestock production systems. They are suitable for very extensive to highly mechanized production systems. They are the most adaptable and geographically widespread livestock species, ranging from the mountains of Siberia to the deserts and tropics of Africa (Luikart *et al.,* 2001). The origin of the domestic goat remains controversial but archaeological evidence suggests that they were probably first domesticated about 10,000 years ago in the Fertile Crescent region of the Near East (Luikart *et al.,* 2001). As a result of both natural selection and selective breeding, certain characteristics in certain populations of goats have been reinforced, giving rise to different goat types and breeds.

The wild ancestors of the present day goat and many other mammalian livestock were of varying colours. However, uniform colour has been established with very intense selective breeding to make coat colour a major breed characteristic. As a result of artificial selection, some coat colours have reached higher frequencies in certain breeds of mammals than others (Norris and Whan, 2009).

Pigmentation of the skin and hair serves a number of valuable functions. Foremost is the photoprotection of underlying tissues from ultraviolet (UV) radiation (Costin and Hearing, 2007; Deng *et al.,* 2009). Recent studies have also shown that melanin not only functions as a sunscreen to absorb UV light and prevent DNA damage, but that its other properties, e.g. as an antioxidant and a radical scavenger, also play important roles in protecting cells from such damage (Costin and Hearing, 2007; Deng *et al.,* 2009). Coat colour in mammals has been associated with productivity and environmental adaptation

(King *et al.*, 1988; Becerril *et al.*, 1993; Acharya *et al.*, 1995; Singh *et al.*, 1997). For example, the light coloured hair coat and darkly pigmented skin found in some cattle in tropical places have helped in the adaptability of these animals to high levels of solar radiation encountered in tropical climates (Acharya *et al.*, 1995). Also, the percentage of white colour on Holstein cows has been reported to have a beneficial impact on milk yield and reproductive traits in regions of high solar radiation due to decreased absorption of incident solar radiation resulting in reduced heat stress (Becerril *et al.*, 1993). Singh *et al.* (1997) reported that the dark coat colour in Indian desert goats serves as an adaptation mechanism to economize feed energy during cool periods. Other performance traits like weight and various other body measurements, such as body length; shoulder width; head width; etc, have been shown to have a relationship with coat colour (Odubote, 1994; Ebozoje and Ikeobi, 1998; Ozoje and Mgbere, 2002). Coat colour has also served as an important form of camouflage and an integral part of social communication and recognition in many mammalian species (Norris and Whan, 2009).

Biochemical and molecular studies of coat colour started a decade ago with much of it in mice because of its use as an animal model (Barsh *et al.*, 2000; Mohanty *et al.*, 2008). Relatively little research have, however, been carried out on other mammals such as cattle, pig, horse and goat. From previous studies, over 120 genes have been identified to be responsible for coat colour in mice (Gutiérrez-Gil *et al.*, 2007).

1.1 Statement of Problem

Coat colour is an easily recognizable trait, making it simple to follow the inheritance from generation to generation. Domestic animals have a valuable resources of coat colour diversity compared to limited variation in natural population, providing excellent models to decipher the genetic basis of coat colour. In mammals, pigmentation is known to be influenced by more than 120 genes. In most mammals other than mice, only a few of these genes have been characterized at the molecular level (Seo *et al.,* 2007). Homologies have been assigned among different mammals in most cases where specific molecular information are absent (Seo *et al.,* 2007). In goats, the coat colour variation has been very poorly explored at the biological and genetic levels. In Nigeria, the diversity of indigenous breeds of goat reported so far is mostly on phenotype and ecological distribution (Akinsoyinu *et al.*, 1981; Blench, 1999; Olopade *et al.*, 2005; Adeyinka and Mohammed, 2006). There is paucity of information regarding the genetics of coat colour in mammals and even less so in indigenous livestock species in Nigeria and Sub-Saharan Africa. The modification of coat colour frequencies by selection or crossing supposes a correct knowledge of biological and genetic aspects of pigmentation. The ability to determine the mechanisms of coat colour inheritance in goats would be highly valuable to the industry allowing precise breeding programmes for desired colours.

There is evidence of random crossbreeding between Nigerian goat breeds, like most African countries (Adebambo *et al.,* 2011). American goat farmers, on the other hand, usually practise controlled breeding (http://goatkingdom.tripod.com/meat-goat-

production). The study of American goats was, therefore, included to compare the results obtained from both populations.

1.2 Aim and Objectives

This study is aimed at investigating the molecular genetics of coat colour in goats. The following are the specific objectives of the study:

1. To investigate the relationship between coat colour and some body traits in Nigerian goats.
2. To identify mutations in some mammalian coat colour genes in goats.
3. To determine the relationship between identified mutations and coat colour differences among Nigerian and some American goat breeds.
4. To investigate any association between identified mutations and some body traits in Nigerian goats.

1.3 Significance of study

Coat colour of livestock species and other domestic animals is significant since relationships have been established with many productive traits. The variation in the intensity of pigmentation in many domestic animals is attributed to geographic and ecological factors as coat colour is known to confer adaptive and ecological advantages. The coat colour of mammals is determined by the interaction of several genes, mutations of which could also lead to several cellular and neurological anomalies, and disease

phenotypes. Knowledge of the molecular genetics of coat colour in goats and other livestock species will not only help breeders in the selection of their herds, but also help to fully understand the underlying mechanisms behind diseases associated with abnormal pigmentation and serve as a basis for research with respect to the treatment and cure for such diseases.

This study is intended to give useful information about the association of coat colour with body dimensions and weight. It is also to provide information on the association of polymorphisms in some coat colour genes and coat colour in Nigerian goats as well as the association of the polymorphisms with specific body dimensions and weight. These pieces of information are expected to be useful for both traditional method of selection and marker-assisted selection of herds for higher productivity.

1.4 Definition of terms

Allele: An alternative form of a gene or a section of DNA at a particular genetic location (locus).

Amplification: The act of making more copies of a specific DNA fragment.

Balancing Selection: This is a type of selection which results in multiple alleles (different versions of the same gene) all being maintained at some frequency within the population.

Breed: A group of organisms having common ancestors and certain distinguishable characteristics.

Coat colour: The overall or main colour of an animal.

Days open: The period between calving and conception in cows.

Deletion: The loss, as through mutation, of one or more nucleotides from a DNA sequence.

Divergence time: The point in geologic time at which two lineages separated from each other.

DNA Sequencing: This is the determination of the precise order of nucleotides in a sample of DNA.

Domestication: This is the process whereby a population of animals or plants, through a process of selection, is changed at the genetic level, accentuating traits that benefit

humans. Humans have brought these populations under their control and care for a wide range of reasons: to produce food or valuable commodities (such as wool, cotton, or silk), for help with various types of work (such as transportation, protection, and warfare), scientific research, or simply to enjoy as companions or ornaments.

Effective number of alleles: The number of equally frequent alleles that would create the same heterozygosity as observed in the population.

Exons: a nucleic acid sequence that is represented in the mature form of an RNA molecule either after portions of a precursor RNA (introns) have been removed by cis-splicing or when two or more precursor RNA molecules have been ligated by trans-splicing.

Fertile Crescent: The **Fertile Crescent**, nicknamed **"The Cradle of Civilization"** due to the birth of various kingdoms within its borders, is a crescent-shaped region containing the comparatively moist and fertile land of otherwise arid and semi-arid Western Asia. In current usage the Fertile Crescent has a maximum extent and a minimum extent. All definitions include Mesopotamia, the land in and around the Tigris and Euphrates rivers. The major nation in this region is Iraq, with small portions of Iran near the Persian Gulf, Kuwait to the south and Turkey in the north. More typically the Fertile Crescent includes also the Levantine coast of the Mediterranean Sea, with Syria, Jordan, Israel, Lebanon and the West Bank. Water sources include the Jordan River. At maximum extent, the Fertile Crescent also may include Egypt and the Nile Valley within it. The inner boundary is delimited by the dry climate of the Syrian Desert to the south. Around the

outer boundary are the arid and semi-arid lands of the Zagros Mountains to the east, the Anatolian highlands to the north, and the Sahara Desert to the west.

F_{IS}: This is the proportional deviation of observed (H_O) from expected heterozygosity (H_S) within subpopulations.

i.e.

$$F_{IS} = \frac{H_S - H_o}{H_S}, \qquad -1 \le F_{IS} \le 1$$

F_{IT}: This is the proportional deviation of observed (H_O) from expected heterozygosity (H_T) in the pooled population.

i.e.

$$F_{IT} = \frac{H_T - H_o}{H_T}, \qquad -1 \le F_{IT} \le 1$$

Frameshift Mutation: an insertion or deletion in the DNA molecule that shifts the normal reading form for translation. This often leads to nonfunctional protein products.

F_{ST}: This is the proportional deviation of overall expected heterozygosity (H_T) from expected heterozygosity in the subpopulations ((H_S).

i.e.

$$F_{ST} = \frac{H_T - H_S}{H_T}, \qquad 0 \le F_{ST} \le 1$$

Gel Electrophoresis: A technique for separating the components of a mixture of charged molecules (proteins, DNAs, or RNAs) in an electric field within a gel.

Gene Cloning - the process of isolating a gene and obtaining multiple copies of it.

Gene: A segment of DNA that is involved in producing a polypeptide chain; it can include regions preceding and following the coding DNA as well as introns between the exons; it is considered a unit of heredity.

Genetic distance: A measure of the allelic substitutions per locus that have occurred during the separate evolution of two populations or species.

Genotype: The combination of alleles, situated on homologous chromosomes, that determines the phenotype of an individual.

Genotyping - This encompasses a range of applications used to analyze genetic differences between individuals or cells.

Haplotype: A specific allelic constitution at a number of loci within a defined linkage block.

Haplotype diversity: This is a measure of the uniqueness of a particular haplotype in a given population.

Insertion: the addition of one or more nucleotide base pairs into a DNA sequence.

Introns: A segment of a gene situated between exons that is removed before translation of messenger RNA and does not function in coding for protein synthesis.

Marker assisted selection (MAS): a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest.

Marker: A genetic trait or segment of DNA that can be identified and tracked. It is a polymorphic genetic character used to follow the transmission of a chromosomal segment in a family or population.

Missense Mutation: a point mutation in which a single nucleotide is changed, resulting in a codon that codes for a different amino acid. This usually changes the activity of the protein.

Negative or purifying Selection: This type of selection acts on genetic variants, reducing the reproductive fitness of organisms and decreasing their frequency until they (usually) disappear completely from the population.

Nm: This is the number of migrant individuals entering a population each generation (*N* is the effective population size of the recipient population and *m* is the rate ofgene flow).

Nonsense Mutation: This is a point mutation in a sequence of DNA that results in a premature stop codon, or a nonsense codon in the transcribed mRNA.

Nucleotide diversity: This is defined as the average number of nucleotide differences per site between any two DNA sequences chosen randomly from the sample population.

Oligonucleotide: A short sequence of DNA synthesized artificially.

Polymerase chain reaction (PCR): An *in vitro* process that yields thousands to millions of copies of desired DNA through repeated cycling of a reaction involving the DNA polymerase enzyme.

Polymorphism: The occurrence of different forms, stages, or types of a characteristic in individual organisms or in organisms of the same species, independent of sexual variations.

Positive Selection: This is the process by which new advantageous genetic variants sweep a population. Positive selection increases the prevalence of alleles that increases the fitness of individuals in a population.

Primer: A short DNA or RNA fragment that anneals to a singled-stranded DNA and to which further nucleotides can be added by DNA polymerase.

Restriction Fragment Length Polymorphism (RFLP): Variation in DNA fragment banding patterns of electrophoresed restriction digests of DNA from different individuals of a species. This is often due to the presence of a restriction enzyme cleavage site at one place in the genome in one individual and the absence of that specific site in another individual.

Silent Mutation: DNA mutations that do not result in a change in the amino acid sequence of a protein. They may occur in a non-coding region (outside of a gene or within an intron), or they may occur within an exon in a manner that does not alter the final amino acid sequence.

Single nucleotide polymorphism (SNP): any polymorphic variation at a single nucleotide (e.g., either C or T).

Tajima's D: A statistical test created by and named after the Japanese researcher Fumio Tajima. The purpose of the test is to distinguish between a DNA sequence evolving

randomly ("neutrally") and one evolving under a non-random process including directional selection or balancing selection, demographic expansion or contraction, genetic hitchhiking, or introgression. A randomly evolving DNA sequence contains mutations with no effect on the fitness and survival of an organism.

Template: A molecule that serves as the pattern for synthesizing another molecule, e.g. a single-stranded DNA molecule that serves as pattern for building a new second strand.

Transition: A substitution mutation in which a purine is replaced by another purine (e.g. A → G) or a pyrimidine with another pyrimidine (e.g. T → C).

Transversion: A base-pair substitution mutation in which a purine is replaced by pyrimidine (e.g. A → C), and vice versa (e.g. T → G).

Wild type - A strain, gene, or characteristic that prevails among individuals in natural conditions, as distinct from an atypical mutant type.

Wright's fixation index (F): This is a test of mating system, i.e. random mating or selective mating. The formula is as follows: $F = 1-(H_{Obs}/H_{Exp})$, where H_{Obs} is the observed heterozygosity and H_{Exp} is the expected heterozygosity assuming random mating. A positive F value indicates some probability of non-random mating while a negative value indicates some probability of random mating.

ABBREVIATIONS

αMSH – Alpha Melanocyte Stimulating Hormone

AHCY - S-adenosylhomocysteine Hydrolase

ASIP – Agouti Signaling Protein

BLAST – Basic Local Alignment Search Tool

BSA – Bovine Serum Albumin

cAMP - cyclic Adenosine Monophosphate

CDA - Colour Dilution Alopecia

COS - an acronym, derived from the cells being **C**V-1 (simian) in **O**rigin, and carrying the **S**V40 genetic material.

DCT – Dopachrome tautomerase

ER – Endoplasmic reticulum

HMM – Hidden Markov Model

ins - Insertion

IPC - Insemination per conception

LYST - Lysosomal Trafficking Regulator

MC1R – Melanocortin 1 receptor MITF - Microphthalmia-associated Transcription Factor MLPH – Melanophilin

PTPRD - Protein Tyrosine Phosphatase Receptor type Delta

QTL – Quantitative Trait Loci

RFLP- Restriction Fragment Length Polymorphism

SNP – Single nucleotide polymorphism

TRP1 - Tyrosinase Related Protein 1

TRP2 - Tyrosinase Related Protein 2

TYR - Tyrosinase

UV – Ultaviolet radiation

VLDLR – Very Low Density Lipoprotein

Receptor WAD – West African Dwarf

2.0 Literature Review

2.1. Mammalian Pigmentation and Melanogenesis

The pigment melanin comprises of two components, the black to brown eumelanin and the yellow to red phaeomelanin (Olson, 1999; Seo *et al.,* 2007; Deng *et al.,* 2009). The ratio of eumelanin to phaeomelanin determines the overall coat colour observed in animals. Melanin is produced within specific membrane bound organelles known as melanosomes, which are produced only by melanocytes (Marks and Seabra, 2001; Soldati and Schliwa, 2006). Melanocytes are usually found at the basal layer of the epidermis, in close proximity to their place of origin, the primitive melanoblasts, with processes extending some distance between epidermal cells. The cell bodies of melanocytes however appear in the super basal layers of the epidermis when melanin formation is stimulated because of exposure to Ultraviolet (UV) rays or hormone such as melanocyte stimulating hormone. Melanoblasts, which are the precursors of the melanocytes, differentiate from a region of the neural tube known as the neural crest (Costin and Hearing, 2007; Seo *et al.*, 2007). A number of genes affect melanoblasts development, survival and migration in the developing embryo and these include: *kit*, SLF (kit-ligand), neuregulin, *MITF* (micropthalmia transcription factor) and *ednrb* – Figure 1 (Seo *et al.*, 2007).

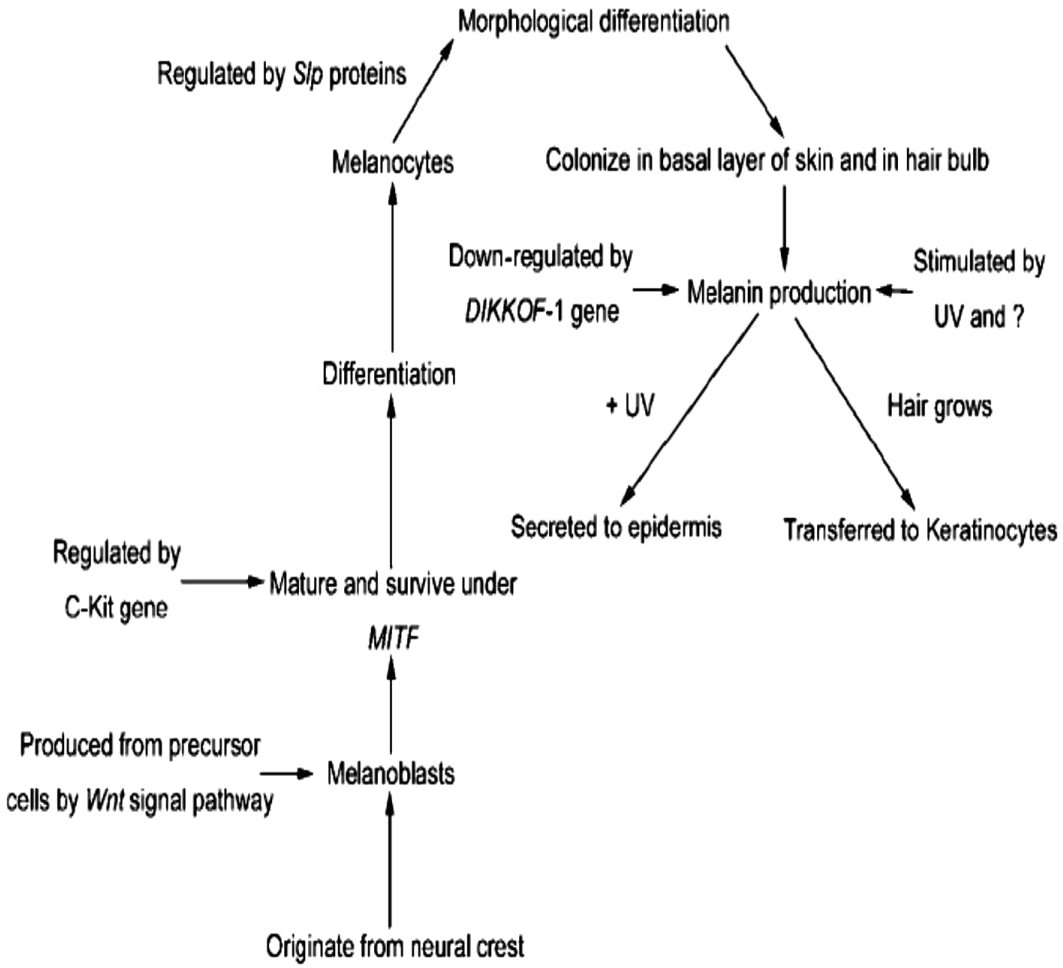

Figure 1: Mechanism of Pigment Formation. Melanoblasts are differentiated from a specialized region of the neural tube, the neural crest. Melanoblasts in turn differentiate into melanocytes under the regulation of growth factors and transcription factors. The protein *MITF* plays a role in the survival and maturation of melanocytes in their early developmental stages. *MITF* increases its transcriptional activity through phosphorylation at serine 73 position under the influence of *c-kit,* a transmembrane receptor with tyrosine kinase activity. Melanocytes undergo further morphological differentiation, under the influence of *Slp* class of proteins, into those that will colonize the basal layer of the skin and those that will colonize the hair bulb. Melanocytes produce melanin when stimulated and melanin production is down regulated, by *DIKKOF-1* gene, when stimulation is ceased.

Source: Seo *et al.,* 2007.

The differentiation of melanoblasts into melanocytes is associated with a change in cell shape from round to stellate with formation of organelles containing melanin granules, melanosomes, through a series of intermediate steps (Costin and Hearing, 2007; Seo *et al.*, 2007). Melanosomes contain melanogenic enzymes and cofactors, including the tyrosinase gene family of proteins; *tyr* (tyrosinase), *trp-1* (tyrosinase related protein-1) and *trp-2* (tyrosinase related protein-2); *pmel17* (silver), *P* (pink eye dilution) and *MATP* (membrane-associated transport protein) (Marks and Seabra, 2001; Seo *et al.*, 2007). The tyrosinase genes form a complex, with the stabilization of *tyr* by *trp-1* and *trp-2*. The synthesis of melanin is greatly influenced by the enzyme tyrosinase whose exit from the endoplasmic reticulum is also under the regulation of calnexin, a protein that promotes proper folding by glycosylation. Tyrosinase is then transported from the endoplasmic reticulum into the Golgi complex, for further processing, and then to the melanosomes where it plays its role in melanogenesis (Marks and Seabra, 2001; Costin and Hearing, 2007; Seo *et al.*, 2007).

The maturation of melanosomes is accompanied by their movement along the microtubules for long distances and actin filaments for short distances (Marks and Seabra, 2001; Soldati and Schliwa, 2006; Seo *et al.*, 2007). Melanosomes bind to the microtubules with the help of the motor proteins kinesins and energy provided by *rab* GTPases (*rab27a*) and to actin filaments by *Slac2*, a *rab27a*-binding protein also known as melanophilin, and *MyoVa. Slac2* controls melanosome distribution in the cell periphery and co-localizes with *rab27a* on the melanosomes. Proper melanosome

transport is also regulated by the C-terminal domain of *Slac2a* (Marks and Seabra, 2001; Soldati and Schliwa, 2006; Costin and Hearing, 2007; Seo *et al.,* 2007).

Eumelanin and phaeomelanin are derived from tyrosine through a series of intermediate steps. Eumelanin, a highly heterogenous polymer, consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) units and the pyrole units derived from their peroxidative cleavage while phaeomelanin consists primarily of sulphur containing benzothiazine. Tyrosine is converted to dopaquinone, a highly reactive intermediate, by the action of tyrosinase. In the absence of thiol compounds, dopaquinone undergoes intermolecular cyclization resulting in the synthesis of eumelanin. The synthesis of phaeomelanin on the other hand begins with the reaction of dopaquinone and a thiol compound, such as cysteine, which results in cysteinyldopas, thiol adducts of DOPA. Cysteinyldopas then undergoes oxidation which leads to the production of phaeomelanin via benzothiazine intermediates – Figure 2 (Sturm and Frudakis, 2004; Hood; 2005; Seo *et al.*, 2007).

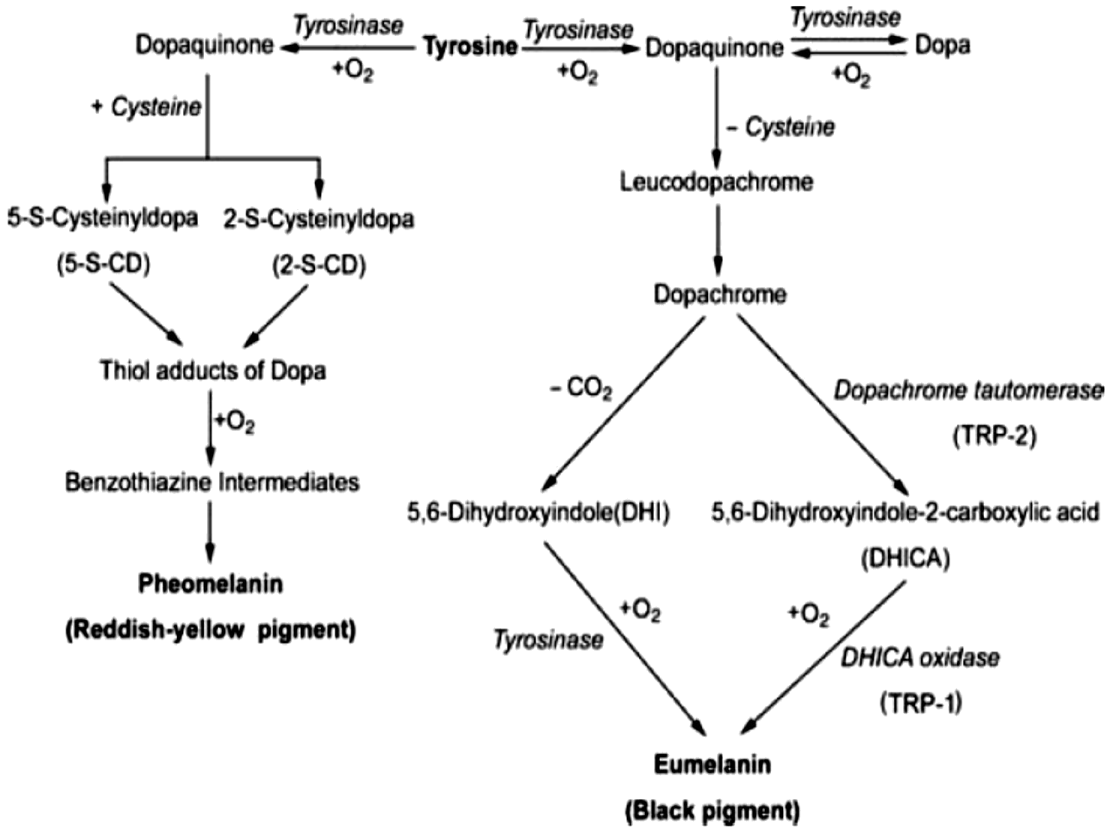

Figure 2: Synthesis of Eumelanin and Phaeomelanin. Eumelanin and phaeomelanin are synthesized from dopaquinone under the influence of the tyrosine family of genes. The synthesis of phaeomelanin is mediated by the conversion of dopaquinone, in the presence of sulphur containing amino acids like cysteine, to thiol adducts and then its oxidation to benzothiazine intermediates which on further oxidation lead to the formation of phaeomelanin. The synthesis of eumelanin on the other hand is mediated by *TRP-2* genes which catalyse dopaquinone to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) which is then converted to eumelanin under the influence of *TRP-1*. Eumelanin can also be produced, less efficiently, from dopachrome via 5,6-dihydroxyindole (DHI) by the oxidative action of tyrosinase.

Source: Seo *et al.,* 2007.

Melanosomes, through their ability to absorb and scatter light and to reduce reactive oxygen species, are important for photo-protection. In response to exposure to sunlight, there is an increase in melanocyte number and subsequent increase in melanosomes and melanin production (Hood, 2005; Seo *et al.*, 2007). A decrease in the intensity of the light rays also brings about corresponding decrease in melanin production. The increased expression and down-regulation of melanin are controlled by *Kit* gene that codes for *c-kit*, a transmembrane receptor with tyrosine kinase activity, and the *DIKKOF-1* gene respectively. DIKKOF-*1* gene codes for *DIKKOF-1* protein which serves in the down-regulation of melanin by inhibiting the *Wnt* signaling pathway - Figure 1 (Hood, 2005; Seo *et al.*, 2007).

The pigmentation of mammalian hair entails the transfer of melanosomes to keratinocytes and a number of views have been expressed explaining how this is accomplished. According to Yamamoto and Bhawan (1994), melanosomes are exocytosed into the extra cellular space and are then taken up by keratinocytes. Seiberg *et al.* (2000) and Sharlow *et al.* (2001) suggested phagocytosis of melanocyte dendrites by keratinocytes. In the study conducted by Scott *et al.* (2002), filopodia, formed by a small GTP-binding protein known as *Cdc42*, were however observed to be the conduits in melanosome transfer to keratinocytes. Scott *et al.* (2002) attributed the discernible increase in melanosome containing filopodia and their attachment to keratinocytes to the expression of constitutively active *Cdc42* proteins found in melanocytes. They found *MyosinVa*, a motor protein which contained actin but not microtubules, at the tips of the filopodia and thus attributed to it a role in melanosome transfer. Melanocytes are known to respond to

keratinocyte derived growth factors by directing filopodia growth and attachment and subsequent transfer of melanosomes to keratinocytes (Seo *et al.,* 2007). According to Matesic *et al.* (2001), a tripartite protein complex of melanophilin, myosinVa and Rab27A is responsible for the transfer of melanosomes in mammals from the cell body to the tips of their dendrites by an actin-dependent movement.

2.2 Genetics of Coat Colour in Mammals

A number of genes have been found to control coat colour in mammals and mutations in these genes result in different shades of colour (Kijas *et al.*, 1998; Ishida *et al.*, 2001; Vrotsos *et al.*, 2001; Candille *et al.*, 2007; Schmutz *et al.*, 2007; Thiruvenkadanr *et al.*, 2008; Dreger and Schmutz, 2010). In cattle for example, several colours and patterns have been observed and these include: black, red or yellow, albinism, brown, roan, spotted, blaze, brockling, colour sided, brindle, premature graying of hair, dilution of the pigment, etc (Olson, 1999; Kantanen *et al.*, 2000; Hillis, 2004; Schmutz, 2010).

The black and red or yellowish colours, observed in many mammals, are mainly determined by the e*xtension* (*E*) and *agouti* (*A*) loci which are responsible for a relative proportion between eumelanin and phaeomelanin (Kijas *et al.*, 1998; Olson, 1999; Schmutz *et al.*, 2003; Hoekstra, 2006; Hirobe *et al.*, 2007; Dreger and Schmutz., 2010). In a number of animals, dominant alleles at the *E* locus produce black coat colour while recessive alleles produce red or yellowish colour. On the other hand, dominant alleles at the *A* locus produce yellow colour while recessive alleles produce black colour (Kijas *et al.*, 1998; Olson, 1999).

Three alleles have been defined for the E locus in cattle: E^D, for dominant black; E^+, responsible for combinations of red or reddish brown and black; and *e*, for recessive red. E^D is dominant to E^+ which is dominant to *e*, i.e. $E^D > E^+ > e$ (Kantanen *et al.*, 2000; Klungland *et al.*, 1995). The *E* locus, which encodes the melanocortin-1 receptor (MC1R) also known as α-melanocyte stimulating hormone (α-MSH) receptor, determines the ratio of eumelanin and phaeomelanin by regulating the level of tyrosinase production (Schaffer and Bolognia, 2001; Scott *et al.*, 2002; Rouzaud *et al.*, 2003). The α-MSH binds to MC1R, thus activating adenyl cyclase via G-protein. The activation of adenyl cyclase leads to an increase in intracellular cAMP which activates tyrosinase (Schaffer and Bolognia, 2001; Scott *et al.*, 2002; Rouzaud *et al.*, 2003).

In the presence of E^D allele, MC1R receptor is active or functional leading to increased tyrosinase synthesis within the melanocytes resulting in a corresponding increase in eumelanin production (Olson, 1999; Rouzaud *et al.*, 2000). Animals with *ee* alleles, however, have nonfunctional MC1*R* receptors which results in the production of red coat colour (Olson, 1999; Rouzaud *et al.*, 2000). The E^+ allele is said to allow phenotypic expression of *A* locus alleles. Cattle that are E^D/E^+ are therefore black, E^+/e are typical red and E^+/E^+ can be of any colour There are four alleles at the *extension* locus in pigs and these include: E^+ for wild type, E^D for dominant black, E^P for black spotting and *e* for recessive red (Ollivier and Sellier, 1992). Andersson (2003) reported seven alleles corresponding to the four phenotypically defined alleles reported in previous studies.

The agouti-signalling protein, coded for by the *A* locus, also influences the expression of the wild type coat pattern by controlling eumelanin synthesis both spatially and temporally. The wild-type *agouti* gene in many mammals acts by temporally suppressing eumelanin synthesis in the hair follicle melanocyte, producing a subterminal band of yellow/red pigment (Robbins *et al.*, 1993). The *agouti* locus also controls the dorsal and ventral differences in pigmentation in some mammals (Duverger and Morasso, 2009; Furumura *et al.*, 1998). In rabbits, the wild type agouti coat colour consists of grey dorsal fur with a much lighter or white ventral area (Fontanesi *et al.*, 2010). Three alleles are known to be responsible for agouti in rabbits: *A*, light-bellied agouti (wild type); a^t, black and tan; *a*, black nonagouti. *A* is dominant to both a^t and *a*, and *a* is recessive to a^t (Fontanesi *et al.*, 2010).

Albinism has been reported in several mammals such as cattle, mice, rats, rabbits and humans. It is inherited as an autosomal recessive trait in most of these cases, and has been attributed to a mutation of the *tyr* (*C*) gene (Oetting *et al.*, 1993; Olson, 1999; Schmidt-Küntzel *et al.*, 2005; Blaszczyk, 2007). In cattle, a frame shift mutation of this gene at residue 316 resulting in a premature stop codon, which leads to the elimination of one of the two copper binding sites in *tyr* gene, is implicated for albinism (Schmutz *et al.*, 2004). In humans, it has been attributed to mutations in the promoter region of the coding sequence of tyrosinase or in other genes, such as pink-eyed dilution (*P*) gene (Durham-Pierre *et al.*, 1994; Steves *et al.*, 1995). In mice, six albino alleles have been reported, and these include: c^{ch} (chinchilla), c^m (chinchilla-mottled), c^r (ruby-eyed dilute), c^h (Himalayan), c^e (extreme dilution), c^p (platinum), c^{44H} (dark-eyed albino) and *c* (albino)

(Searle, 1990). In rabbits, four alleles are reported at the *C* locus, and these include: c^{+}, c^{ch}, c^{h}, and *c*. The allele c^{+}; which is responsible for full colouration; is dominant to c^{ch} (chinchilla); responsible for the suppression of colour in the intermediate band of the coat. c^{ch} is dominant to c^{h} (Himalayan); responsible for the black colouration of only the hair at the body extremities, and c; responsible for albinism; is recessive to c^{h}, i.e. $C>c^{ch}>c^{h}>c$ (Nussbaum, 1988).

Genes at the *B* locus determine brown coat colour in cattle, rabbit, dog and some other mammals (Barsh, 2001; Berryere *et al.*, 2003). This gene is found to be responsible for *trp-1* protein, which appears in the melanin synthesis pathway after the branch point between eumelanin and phaeomelanin synthesis. It is therefore thought to affect coat colours associated with eumelanin (Berryere *et al.*, 2003). The dilutions of coat colour in varying shades that affect the black and red pigment to the same degree in mammals has been attributed to defects in the transfer of melanosomes from melanocytes to keratinocytes (Wu *et al.*, 1997; Bahadoran *et al.*, 2001). According to Matesic *et al.* (2001) and Ménasché *et al.* (2003), among the three candidate genes, *MLPH*, *Rab27a* and *MyoVa*, associated with this transfer, a mutation in the *MLPH* gene was found to be responsible for colour dilution without any additional impairment in human Griscelli Syndrome 3 (GS3) patients or in leaden mice. The *MLPH* gene was thus considered the gene responsible for colour dilution.

The roan colour seen in some breeds of cattle; like Shorthorns, Belgian Blues and Texas Longhorns; depends on alleles at the *R* locus. Animals heterozygous for *R* (*R/r+*) exhibit

roan or blue colour, homozygous *R* (*R/R*) animals are all-white while $r^{+}r^{+}$ are all black or red (Hanset, 1985; Charlier *et al.*, 1996).

White spotting pattern, observed in some breeds of cattle, depends on *S* or *W* locus that has four alleles; S^{+} (non-spotting), S^{H} (homozygous), S^{p} and *s*; in the order of dominance $S^{H} = S^{p} > S^{+} > s$ (Rooney *et al.*, 1990; Grosz and MacNeil, 1999; Olson, 1999). The S^{H} is seen in Herefords (S^{H}/S^{H}), S^{P} is responsible for lined back pattern seen in Pinzgauers and *s* (recessive spotting) is responsible for the irregular white spotting seen in breeds like Holstein and Guernsey (Rooney *et al.*, 1990; Olson, 1999). The death of individual melanoblasts due to *ednrb* mutations has also been attributed to the different amounts of spotting located in different regions of the bovine and canine body (Rooney *et al.*, 1990; Grosz and MacNeil, 1999; van Hagen *et al.* 2004).

In contrast to the spotting pattern produced by S^{p} genes, where the edges of spots produced are clearly defined, the *Cs* gene produces ragged or roan-like edge spots (Rooney *et al.*, 1990; Olson, 1999; Hillis, 2004). The *Cs* gene is responsible for the colour sided pattern seen in some breeds of cattle like Texas Longhorn, Florida Cracker and English Longhorn (Rooney *et al.*, 1990; Grosz and MacNeil, 1999; Olson, 1999; Hillis, 2004). Animals heterozygous for *Cs* possess white stripping along the dorsal and ventral parts of the animal, with roan areas along the eyes and a roan or dappled pattern of white on the head while the homozygous *Cs* animals possess white coat with pigmented ears and muzzle and sometimes pigmentation above the feet (Olson, 1999; Hillis, 2004). Another white spotting pattern observed in cattle is the blaze pattern,

characterized by the white spotting pattern on the face, which is determined by the *Bl* gene (Olson, 1999; Grosz and MacNeil, 1999; Hillis, 2004). This pattern is observed when the gene is heterozygous in combination with S^{+} (Olson, 1999).

Other spotting patterns observed in cattle include brockling and brindle patterns (Rooney *et al.*, 1990; Grosz and MacNeil, 1999; Olson, 1999). The brockling pattern interacts with apparently any white spotting mutant, producing areas of pigmentation that would normally be white in the absence of the brockling (*Bc*) gene. This pattern has been observed in the offsprings of the cross between Hereford and Angus calves where *Bc* from Angus produces pigmented spots on the face of the progeny (Olson, 1999). The brindling pattern is characterized by narrow alternating stripes of black and red arranged vertically on the entire body or confined to the head, neck and rear quarters. This pattern is due to the presence of the brindling gene and it requires the base colour of the body to be of wild type (i.e. E^{+}/E^{+}, $E^{+} > e$ or *ee*) (Olson, 1999).

Some breeds of cattle have been observed to have a change of colour as they age. A typical example is the Holstein breed where a change of colour from red to black and less commonly from black to red has been reported as the animal ages (Seo *et al.*, 2007). Also, some calves of the Hanwoo breed from South Korea are known to be born with yellowish-brown coat colour but experience a change to blackish colour as they age (Seo *et al.*, 2007). A number of genes have been implicated in the switching of pigmentation, and these include: *MC1R, A, attractin* (*Atrn*) and *Mgrn1* (Barsh *et al.*, 2000; Seo *et al.*, 2007). However, the cause for the switching of pigmentation remains obscure and studies

on cell signaling that leads to switching in pigmentation pattern are in a primitive stage (Barsh *et al.*, 2000; Seo *et al.*, 2007).

Some other genes have been implicated in pigmentation in humans. These include: adrenocorticotropin (*ACTH*) and *β-MSH* (Abe *et al.*, 1969; Spencer *et al.*, 2005; Rousseau *et al.*, 2007; Spencer and Schallreuter, 2009). Lerner and McGuire (1961) observed discernible skin darkening in volunteer individuals upon systemic application of *α*- and *β-MSH*. The studies of Abdel-Malek *et al.* (1995), Hunt *et al.* (1994) and Kauser *et al.* (2003) demonstrated that *ACTH*, *α-MSH* and *β-endorphin* induce melanogenesis, proliferation of epidermal and hair follicle melanocytes, and regulate cell dendricity. *β-MSH* has been implicated in the underlying mechanism governing changes in melanocyte morphology, growth rates and melanin production. Its mode of action is however still obscure (Spencer *et al.*, 2005). This is inferred from the absence of *β-MSH* in melanosome containing *α-MSH*, *β-endorphin*, *proopiomelanocortin* (POMC) processing machinery, *prohormone convertases 1/2* (*PC1, PC2*) and *7B2* in epidermal and hair follicle melanocytes in humans (Spencer *et al.*, 2005).

2.3 Studied genes

2.3.1 Melanocortin-1 Receptor (*MC1R*) gene

The melanocortin 1 receptor (MC1R) is expressed in a variety of cells, including melanocytes and keratinocytes, and functions as a receptor for α–melanocyte stimulating hormone and adrenocorticotropin hormone (*ACTH*). MC1R is a G protein-coupled

receptor of 45 kDa with seven transmembrane domains (Rouzaud *et al.*, 2000; Fontanesi *et al.*, 2009a). As stated earlier, binding of α-melanocyte stimulating hormone with MC1R activates the intracellular cAMP pathway and, through a series of downstream events involving the transcription of tyrosinase gene, leads to the production of the brown/black photoprotective eumelanin (Schaffer and Bologna, 2001). Variations or polymorphisms of the MC1R gene may result in decreased receptor function, either at the level of α-melanocyte-stimulating hormone binding or at the level of cAMP signaling, resulting in a quantitative shift of melanin synthesis from eumelanin to the red–yellow phaeomelanin – Figure 3 (Valverde *et al.*, 1995; Box *et al.*, 1997; Sturm *et al.*, 2001).

A strong relationship between melanocortin-1 receptor gene (*MC1R*) variants and coat colour has been found in several mammals. In humans, more than 77 human alleles of the *MC1R* gene with nonsynonymous changes have been identified to date (Wong and Rees, 2005). Three variants (Arg151Cys, Arg160Trp, and Asp294His), in particular have been defined as ‘‘red hair colour’’ variants based not only on their diminished receptor function *in vitro*, but also on their significant association with specific phenotypic features, such as red hair, fair skin, and freckling (Box *et al.*, 1997; Rana *et al.*, 1999; Flanagan *et al.*, 2000; Bastiaens *et al.*, 2001; Duffy *et al.*, 2004). Box *et al.* (1997) and Rana *et al.* (1999) found the Arg151Cys to be associated with the red-hair/fair skin. MC1R with this variant was observed to show lower response to NDP-MSH, a potent synthetic analogue of α-MSH, which is consistent with this variant associating with phaeomelanin-rich skin.

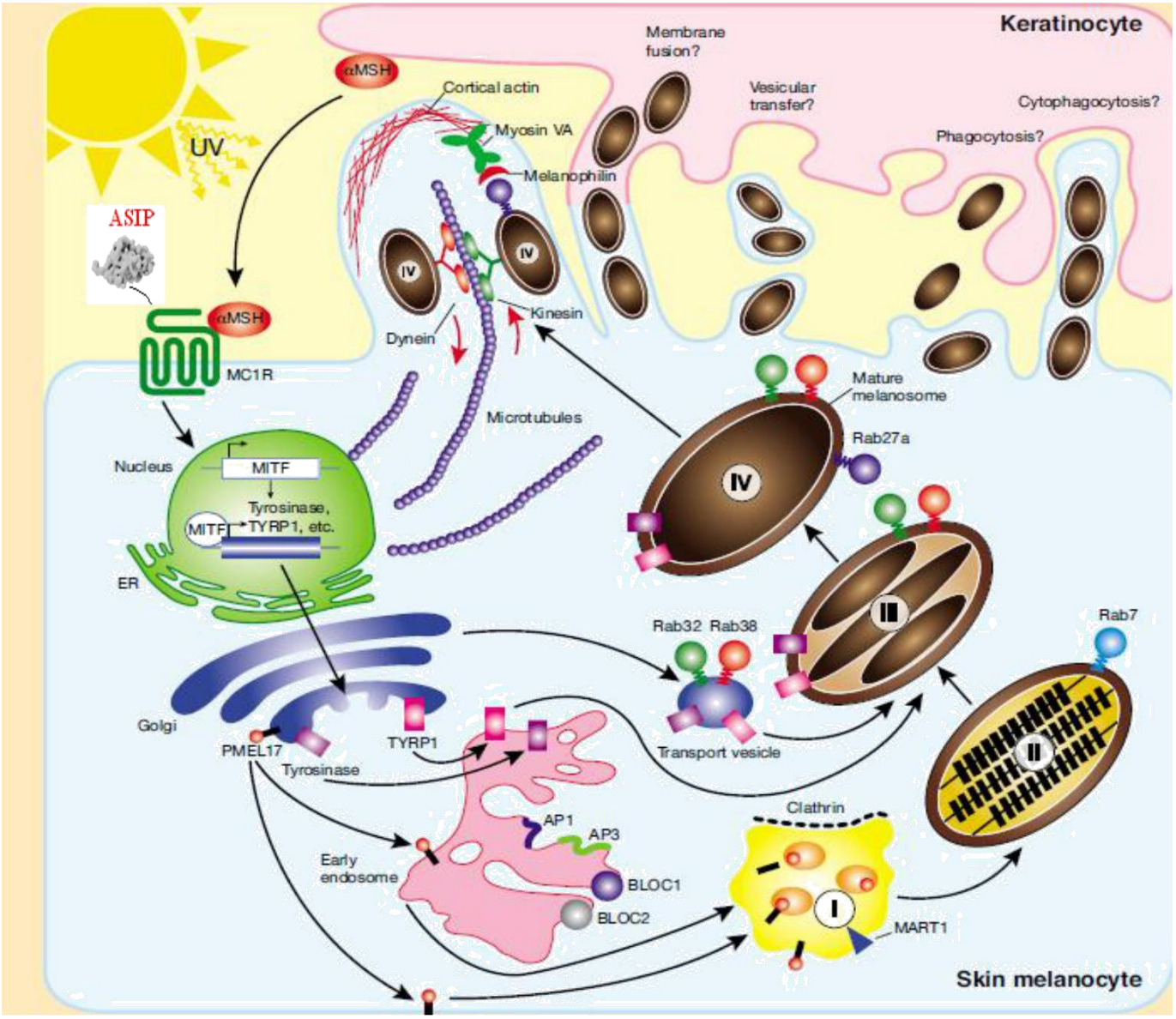

Figure 3: Regulation of melanogenesis in skin melanocytes. The synthesis of melanin, in particular black eumelanin, is stimulated primarily by ultraviolet (UV) irradiation, which generates DNA photoproducts and leads to the release of various autocrine and paracrine factors, of which the most notable – α-melanocyte-stimulating hormone (αMSH) is secreted by keratinocytes. αMSH activates the melanocortin 1 receptor (**MC1R**) in the plasma membrane of skin melanocytes, which results in cAMP-dependent signalling and the stimulation of the expression of microphthalmia-associated transcription factor (MITF), a 'master regulator' of melanocyte function and melanogenesis. The agouti signaling protein (**ASIP**), a paracrine signaling molecule, however acts as an antagonist of αMSH, blocking the production of cAMP and thus leading to a pigment-type switching from eumelanins to phaeomelanins. Melanosomes gradually develop melanin as they mature and are translocated from the perinuclear region to the periphery (dendrites) of the melanocytes where they are then transferred to neighbouring keratinocytes in the middle and upper layers of the skin. Melanosomal transfer and translocation are driven by two distinct motors, a microtubule-dependent motor and an actin-dependent movement. Mature melanosomes move along microtubules from the perinuclear region of the melanocyte towards the cell periphery through an interaction with kinesin. The transfer of melanosomes to keratinocytes is then accomplished by the recruitment of **melanophilin** by Rab27A located on the surface of mature melanosomes (Wasmeier *et al.*, 2008).

Other alleles, such as Val92Met, Val60Leu, 86insA, Asp84Glu, Arg142His, Ile155Thr, and His260Pro, have been statistically considered as full or partial ‘‘red hair colour’’ causing variants, based on genetic association and family studies (Valverde *et al.,* 1995; Valverde *et al.,* 1996; Rana *et al.,* 1999; Sturm, 2002). Rana *et al.* (1999) reported no variation in *MC1R* in African populations as the human consensus protein sequence was observed in all the 25 African individuals studied. One of the variants they reported, Arg163Gln, was absent in the Africans studied, almost absent in Europeans, and at a low frequency (7%) in Indians, but at an exceptionally high frequency (70%) in East and Southeast Asians. Peng *et al.* (2001) also found this variant, Arg163Gln, to be common among four Chinese ethnic populations.

In cattle, Sasazaki *et al.* (2005) identified three alleles at the *MC1R* locus in Japanese Black, Japanese Brown and Hanwoo (brown/red) cattle: the wild type E^+ allele which has a coding sequence of 954 bp and 317 amino acids, the E^D allele with a single base substitution (T296C) leading to an amino acid change from leucine to proline, and the *e* allele which has a deletion of guanine at position 310, leading to a frameshift mutation and a predicted protein of 155 amino acids. From their results, Japanese Black had no homozygous *ee*, they were composed of E^D and E^+ individuals, E^D = 0.481 and E^+ = 0.514. This is consistent with the theory that E^D and E^+ alleles allow α-melanocyte stimulating hormone to bind to MC1R leading to black pigmentation. Most of the animals belonging to the Hanwoo breed were *ee* as expected. In general, Japanese Brown and Hanwoo were E^+/E^+, *e*/*e* or E^+/e. However, allele frequencies differed considerably between the two breeds. The wild type E^+ allele frequency was 0.962 for Japanese Brown

and 0.052 for Hanwoo. According to Klungland (1995), the E^+ allele allows the phenotypic expression of *A* locus alleles. E^+ animals therefore produce brown coat colour in the presence of A^+ allele at the *A* locus and recessive black coat colour in the presence of homozygous *a* alleles.

Klungland *et al.* (1995) also detected two mutations in the *E*-locus in cattle, upon sequencing PCR products, from black, brown, and red coat-coloured animals from Norwegian cattle breeds, and Icelandic cattle. All three coat colours were present in both populations of animals studied. Compared with the wild type allele E^+, they found one single base substitution resulting in an amino acid change from leucine to proline in almost all black animals (allele E^D). A single base deletion, leading to a frameshift, was found in black, brown, and red animals (allele *e*). Homozygous *e/e* animals were found to be always red. In addition to the dominant black colour caused by the *E* allele, a recessive black colour was found in the Icelandic cattle population. Among nine black Icelandic cattle, only seven were found to have the dominant allele E^D. The remaining two animals possessed the genotype E^+/e and E^+/E^+, respectively. According to them, alleles at the *A*-locus might be responsible for the recessive black colour. They also found several red binanimals in both cattle populations with the genotype E^+/e *or* E^+/E^+. They proposed that the wild-type receptor encoded by the E^+ allele is probably inhibited in these animals and that a dominant *A*-locus allele blocking the MSH-receptor could explain this colour switch from the expected brown or black to red. In addition to the three alleles reported by Klungland *et al.* (1995) and Sasazaki *et al.* (2005), Rouzaud *et al.* (2000) reported a fourth allele named E^1, characterized by a 12 bp duplicated sequence at position 669

which leads to a four amino acid (gly-ile-ala-arg) duplication within the third intracellular loop of the receptor, in French cattle breeds. These additional amino acids can induce a modification of the third loop conformation consequently changing interactions with the corresponding G protein and in the signal transmission resulting in the agouti and/or α-MSH binding. These molecular changes might be the reason why animals with this allele had attenuated coat colour (Rouzaud *et al.,* 2000).

Fontanesi *et al.* (2009a) sequenced the whole coding region of the *MC1R* gene in goats of six different breeds showing different coat colours (Girgentana, white cream with usually small red spots in the face; Maltese, white with black cheeks and ears; Derivata di Siria, solid red; Murciano-Granadina, solid black or solid brown; Camosciata delle Alpi, brown with black stripes; Saanen, white; F1 goats and the parental animals). They identified five single nucleotide polymorphisms (SNPs): one frameshift mutation (p.Q225X), three missense mutations (p.A81V, p.F250V, and p.C267W), and one silent mutation. The frameshift mutation at position 225 should cause the production of a shorter MC1R protein whose functionality may be altered. The Girgentana breed was almost fixed for the p.225X allele. However, there was no complete association between the presence of red spots in the face and the presence of this allele in homozygous condition. The same allele was identified in the Derivata di Siria breed. However, its frequency was only 33%, despite the fact that these animals are completely red. The p.C267W allele was present in all Murciano-Granadina black goats, whereas it was never identified in the brown ones. Moreover, the same substitution was present in almost all Maltese goats providing evidence of association between this mutation and black coat colour. The result of

Fontanesi *et al.* (2009a) indicates that *MC1R* mutations determine eumelanic and pheomelanic phenotypes. However, they are probably not the only factors. In particular, the incomplete association of the nonsense mutation (p.Q225X) with red coat colour raises two hypotheses: i) the presence of a second "red allele" at the *MC1R* locus; and ii) the role of an additional gene (probably different from *ASIP*).

2.3.2 Agouti Signaling Protein (*ASIP*) gene

The *Agouti* locus encodes the agouti signaling protein (ASIP), a paracrine signaling molecule that acts as an antagonist of αMSH (contrasting the hormone-receptor interaction), blocking the production of cAMP and thus leading to a pigment-type switching from eumelanins to phaeomelanins – Figure 3. The mouse *agouti* gene encodes a 131 amino acid protein normally expressed in the skin during hair growth (Bultman *et al.*, 1992). The coding region of the *ASIP* gene, encoded by 3 exons (2, 3, and 4, according to the nomenclature in mouse), has been completely sequenced in a number of mammals with a predicted protein of 131–135 amino acids. The ASIP protein sequence in mice consists of a 22 amino acid secretion signal sequence, a highly basic amino-terminal domain present at the center (Lys57-Arg85), a polyproline stretch, and a cysteine-rich carboxy-terminal domain (Cys92-Cys131) that fold in a characteristic knot structure. The cysteine-rich domain is responsible for melanocortin binding activity: Val83, Arg85, Pro86, and Pro89 are important for inhibition of binding to MC3-R, MC4-R and MC5-R while Val83 is also important for inhibition of MC1-R (Kiefer *et al.*, 1997). Kiefer *et al.* (1997) observed 5-15 fold and 100-500 fold increase relative to wild

type in inhibition dissociation constant ($K_{I\ app}$) values for both binding and cAMP production for Δbasic (Δ57-85) and ΔC-term (truncated agouti without carboxyl-terminal sequence), respectively. More than 50 alleles have been observed at the a*gouti* locus in mice with most having been characterized at the DNA level (Fontanesi *et al.,* 2009b). Mutations at this locus associated with coat colours have also been identified in other species. These mutations can affect both non-coding and coding regions (Fontanesi *et al.,* 2009b).

Fontanesi *et al.* (2009b) investigated the association of coat colour with *ASIP* gene in 6 goat breeds with different coat colours and patterns: Girgentana, Maltese, Derivata di Siria (also known as Rossa Mediterranea or Mediterranean Red), Murciano-Granadina, Saanen and Camosciata delle Alpi. Their studies revealed a protein of 133 amino acids; a signal sequence of 22 amino acids followed by a basic amino-terminal domain of 65 residues (containing 19 lysines or arginines), 4 prolines, and a 42-residue cysteine-rich carboxy-terminal domain. They identified 5 SNPs: one in intron 3 (g.5G 1 T) and four in exon 4. Of the four exonic mutations, three are missense mutations (p.Ala96Gly; p.Cys126Gly; and p.Val128Gly) and are located in highly conserved positions of the mammalian ASIP carboxy-terminal domain. Only the p.Val128Gly seemed to probably play a role in the dark coat colour of the Murciano-Granadina breed together with a mutation in the *MC1R* gene. Similar mutations have been found in dogs. Kerns *et al.* (2004) found a missense mutation at position 96 (p.Arg96Cys) in German shepherd dogs and the presence of this additional cysteine in the carboxy-terminal domain was thought to alter the functionality of the protein hence the black nonagouti coat colour phenotype.

The p.Ala96Gly mutation found in goats by Fontanesi *et al.* (2009b), however, did not seem to have any putative major change in protein conformation. They also identified Copy Number Variation (CNV) affecting a region of less than 100 kb including the *ASIP* and S-adenosylhomocysteine hydrolase (*AHCY*) genes. According to them, this CNV might cause the A^{Wt} allele, as suggested for a similar structural mutation in sheep affecting the *ASIP* and *AHCY* genes, providing evidence for a recurrent interspecies CNV.

Tang *et al.* (2008) also identified a missense mutation, Gly125Val, at position 423 in exon 4 in Chinese indigenous goats. They identified two alleles, T and G, and three genotypes, TT, TG and GG. Goat breeds with black coat colour were found to have a higher frequency for the T allele. They concluded that allele T might be responsible or linked with the causative site for the black phenotype in the studied goat breeds. Voisey *et al.* (2006) also observed a strong association between the G allele and dark hair colour in humans for the g.A8818G mutation in the 3' Untranslated Region (UTR) of *ASIP*, resulting from decreased levels of ASIP and hence less phaeomelanin production. In horse, Rieder *et al.* (2001) found complete association between an 11 bp deletion in exon 2 of *ASIP* and recessive black coat colour. Våge *et al.* (1997) also found a deletion in exon 1 of the *agouti* gene to be associated with a proposed recessive allele of *agouti* in the darkly pigmented Standard Silver fox (*aa*). Royo *et al.* (2005) and Girardot *et al.* (2006), however, found no evidence of coding-region sequence variation within and between nine wild type and eight cattle breeds respectively, representing a large panel of coat colour phenotypes.

2.3.3 Melanophilin (*MLPH*) gene

Melanosomes gradually develop melanin as they mature and are translocated from the perinuclear region to the periphery (dendrites) of the melanocytes where they are then transferred to neighbouring keratinocytes in the middle and upper layers of the skin. Skin and hair colour of many mammals are determined by the form, contents, transfer and accumulation of melanosomes in keratinocytes. More deeply-pigmented skins usually contain larger and higher number of melanosomes when compared to light-pigmented skins (Sturm, 2006). Skin tanning in response to ultraviolet radiation, therefore, results in an increase in melanocyte number and an increase in the transfer of melanosomes from melanocytes to keratinocytes (Sturm, 2006; Costin and Hearing, 2007).

Mammalian pigmentation is controlled by several genes whose products form the melanosomal particles or are involved in their maturation and dispersion. The movement of melanosomes in melanocytes, whose defect leads to the three types of Griscelli syndrome in humans, the ashen, leaden and dilute phenotype in mice, and the dilute coat colour phenotype in several animals is controlled by three major proteins, which include: myosin VA (MYO5A), Rab27A and melanophilin (MLPH), (Fukuda *et al.,* 2002; Drögemüller *et al.,* 2007; Aspengren *et al.,* 2009). Melanosomal transfer and translocation are driven by two distinct motors, a microtubule-dependent motor and an actin-dependent movement (Goud, 2002; Aspengren *et al.,* 2009). Mature melanosomes move along microtubules from the perinuclear region of the melanocyte towards the cell periphery through an interaction with kinesin (Goud, 2002; Aspengren *et al.,* 2009). The transfer of melanosomes to keratinocytes is then accomplished by the recruitment of

melanophilin by Rab27A located on the surface of mature melanosomes – Figure 3 (Drögemüller *et al.*, 2007). Melanophilin acts as a linker protein between melanosomes and myosin Va-bound actin filament by binding the GTP-bound form of Rab27A on melanosomes via its N-terminal synaptotagmin-like protein (Slp) homology domain and myosin Va, via one of its C-terminal coiled-coil domains (Fukuda *et al.*, 2002; Goud, 2002; Nagashima *et al.*, 2002; Hume *et al.*, 2006). This interaction between melanophilin and myosin Va is strengthened by the presence of a melanocyte-specific exon F in the tail domain of myosin Va (Fukuda *et al.*, 2002; Goud, 2002; Hume *et al.*, 2006).

Mutations in the melanophilin gene have been associated with coat colour dilution in some mammals. Philipp *et al.* (2005) reported strong associations for single nucleotide polymorphisms in exon 2 of *MLPH* in dilute Doberman pinschers, Beagles, and Large Munsterlanders and in exon 7 in dilute German pinschers. Drögemüller *et al.* (2007) sequenced the 5' region of the canine *MLPH* gene using 285 purebred individuals from 7 dog populations segregating for the dilute coat colour phenotype (131 German pinschers, 109 European Doberman pinschers, 15 American Doberman pinschers, 13 Rhodesian Ridgebacks, 7 Beagles, 6 Large Munsterlanders, and 3 Miniature pinschers) and a single dilute and Colour Dilution Alopecia (CDA)-affected American Staffordshire. They observed three different dilute associated haplotypes, two of which were exclusively found within Doberman pinschers of American origin (d2) and German pinschers (d3), respectively. The third dilute haplotype (d1) was found in Beagles and Doberman pinschers from European ancestry. The three different dilute haplotypes shared a 7.8 kb segment of identical alleles and this region was presumed to harbor the causative dilute

mutation. They compared the dilute associated haplotypes in this candidate interval and the observed wild-type haplotypes and found 14 polymorphic sites (11 SNPs, 2 indels, and 1 microsatellite) that were perfectly associated with the dilute phenotype. The likelihood of these polymorphisms to be causative was ranked based on their position within the *MLPH* gene. Among the 14 associated polymorphisms was an A/G SNP located at the last nucleotide of the untranslated exon 1 (c.22G>A). This SNP was thought to have an effect on splicing efficiency and was considered a strong candidate SNP. Genotyping of this SNP in 285 animals revealed perfect association with the dilute phenotype. This SNP also showed perfect cosegregation with the dilute phenotype in several 2-generation families. To evaluate the effect of this SNP on splicing efficiency, expression study using quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) was carried out. Their results confirmed that *dd* animals had only about approximately 25% of the *MLPH* transcript compared with *DD* animals.

Zhou *et al.* (2010) investigated the association of a mutation in *MLPH* with coat colour in 304 goats belonging to nine Chinese goat breeds distributed in Hebei, Sichuan, Shandong, Hainan and Liaoning provinces, and Chongqing city in China. They identified a missense mutation g.*115844A>G* in exon 10 in melanophilin gene upon sequencing PCR products from eight individuals. This SNP was further genotyped in the 304 goats using PCR-RFLP. From their results, allele A was found to be predominant while frequencies of allele G ranged from 0 to 39.58%. The four breeds/strains found in Sichuan province and Chongqing city, which are adjacent in the Western mountain area in China, had the highest G frequencies. They include: Chengdu Ma goat with 39.58%,

Nanjiang Brown goat (Fast growing strain) with 29.41%, Nanjiang Brown goat (High fertility strain) 27.14%, and Nanjiang Brown goat (Black strain) 26.53%. They found homozygous GG only in these four breeds/strains, indicating that the allele G might be a recessive site responsible for the specific brown coat colour of Chengdu Ma goat and Nanjiang Brown goat.

Li *et al.* (2010) sequenced amplified fragments of the melanophilin gene in 108 Chinese goats belonging to 5 populations. They detected nine SNPs, named following the sequence of *MLPH* gene (382bp) deposited in GenBank with accession number EU195227, four in intron 7 (90 A>T, 97C>A, 120A>G, and 155C>T), and 5 in exon 8 (241C>G, 253G>A, 263G>A, 275C>T, and 292C>T). Among the five SNPs in exon 8, two were synonymous mutations, at positions 263 and 275, while three were missense mutations at positions 241(Ala→Gly), 253(Arg→His), and 292(Pro→Leu). They found only two haplotypes, ACACCGGCC represented as A and TAGTGAATT represented as B with frequencies of 92.59% and 7.41% respectively. They also detected only two genotypes in all individuals, AA and AB with frequencies of 85.19% and 14.81% respectively. According to these authors, the nine SNPs are probably linked and allele B might be linked with a recessive lethal gene. Additionally, they found that allele B was not found in Chengdu Ma goat and deduced that the absence of allele B might be related to the special dilute coat colour (tan) of Chengdu Ma goat.

2.4 Coat Colour and Performance Traits

Hair coat characteristics, particularly colour, are known to have an influence on reproductive performance as well as on milk production in regions of high solar radiation. King *et al.* (1988) using data from a Holstein herd in Arizona for which sprinklers and shade areas were provided found the interaction between coat colour and season of freshening to be significant for days open and services per conception. White cows freshening in February and March required fewer services per conception and had fewer days open than the mixed and black cows. Becerril *et al.* (1993) also reported positive regression coefficients for reproductive traits in association with white coat colour of Holstein cows during the summer months in Florida, but their values were not statistically significant. However, Bertipaglia *et al.* (2005) found a decrease in Insemination per conception (IPC) with increase in the percentage of black hairs. The deviation of their result from the expected higher number of IPC of cows with more than 70% black hairs is probably due to the fact that the cows were managed under shade structures, protected against the direct solar radiation, and provided with fans and sprinklers. Bertipaglia *et al.* (2005) hypothesized that predominantly black cows have a greater absorption of thermal radiation which leads to increased coat surface and rectal temperatures which consequently decreases conception rate.

Hansen (1990) found a relationship between colour and environment (shaded and unshaded areas) for physiological variables for Holsteins grouped by percentage of white hair. He observed that cows with white hair, exposed to sun without shade, had smaller

changes in their physiological variables and less depression in milk yield. In agreement with Hansen's (1990) observation, Maia *et al.* (2005) reported that milk yield in predominantly (>70%) white-haired cows was found to be higher than in predominantly black cows. Schleger (1967) also observed an increase in milk yield with a decrease in the intensity of pigmentation.

Other productivity traits like weight and various other body measurements, such as body length; shoulder width; head width; etc, have been shown to have a relationship with coat colour. Ozoje and Mgbere (2002) studied the effect of coat pigmentation on live-weight and other body measurements in West African Dwarf (WAD) goats. They reported that coat pigmentation significantly affected live-weight with the white and or tan goats having the heaviest weights. Coat pigmentation was found to have no significant effect on body dimensions studied except leg length. Frame size was also found to increase with decrease in coat pigmentation intensity. They observed that the white and or tan goats had the largest frame size followed by brown goats. These white and or tan goats, however, had shorter legs when compared with either the brown or black goats. Odubote (1994) also observed that body weight of post-weaned kids increased with decreasing level of pigmentation. Ebozoje and Ikeobi (1998) reported that prolificacy, fecundity and litter size at birth and at weaning were significantly affected by coat colour in West African Dwarf goats. Contrary to the report of Odubote (1994), Ebozoje and Ikeobi (1998), and Ozoje and Mgbere (2002) observed that the age at first kidding and birth weight increased with pigmentation intensity. A significant difference in live weight, based on genotype and coat colour, was also observed in cattle in the study conducted by

McClean and Schmutz (2009). Black cattle (E^D/E^D or E^D/e) were found to be significantly heavier than red cattle (*ee*). The association of coat colour with body weight, according to McClean and Schmutz (2009), is probably due to a reduction in the amount of α-MSH binding to MC4R, an appetite inhibiting receptor. Animals with at least one E^D allele have functional MC1R, another receptor for α-MSH, thereby reducing the amount of α-MSH available to bind to MC4R. Another speculation is linkage disequilibrium of the *MC1R* mutation with mutation in another gene that causes weight and fat deposition differences in cattle (McLean and Schmutz, 2009).

The studies of Gratten *et al.* (2008) on the free-living Soay sheep of St. Kilda, Scotland revealed that coat colour is associated with body size and fitness. The authors noted that variation in coat colour in these sheep is controlled by a single autosomal locus with the dark allele been dominant to the light allele and that homozygosity of a single recessive amino acid substitution at coding position 869 in the *tyrosinase related protein 1 (TRP1)* gene gives rise to the light phenotype. They observed a strong genetic association between *TRP1* and birth weight. Sheep with genotypes GG and GT (dark coats) were found to be heavier at birth than sheep with genotype TT (light coats). Sheep with genotype GG were found to be largest but not significantly larger than dark heterozygotes (GT) while they also recorded a significant genetic association between *TRP1* and fitness. The relationship between coat colour and fitness was, however, found to be inconsistent with that of body size and that homozygous dark sheep (GG) showed evidence of reduced fitness relative to heterozygous dark sheep (GT). On the other hand, no evidence for differential fitness was observed between heterozygous dark sheep (GT) and light sheep

(TT). A plausible reason for the association of coat colour with body size and fitness is linkage disequilibrium with some body size and fitness QTLs. These scientists similarly reported the linkage of *TRP1* with *Very Low Density Lipoprotein Receptor* (*VLDLR*), a gene associated with neonatal variation, and *Protein Tyrosine Phosphatase Receptor type Delta* (*PTPRD*), a fitness associated phenotype determining gene, in mice.

2.5 Coat Colour and Environmental Adaptation

Various researchers have conducted experiments to verify the importance of coat colour on adaptation and in most of these cases, correlations were found to exist. In a research on the sweating rates of three lines of cattle, Gebremedhin *et al.* (2008) found that white hair coat resulted in significantly lower dorsal skin temperature relative to black hair coat. When the animals were challenged in an environment of increasing solar load and wind speed, the white animals responded linearly with higher sweating rates. Archarya *et al.* (1995) also exposed Sirohi female goats to more than 8 hours of sun per day for 5 days in May, the hottest month in summer, in a bid to establish the relationship between heat tolerance and coat colour. They found that the rectal temperatures, respiration rates and pulse rates were highest in black, followed by dark brown, light brown and then white goats, in descending order. Thus, white coat colour has an advantage over dark coat colour. Knowledge of physics demonstrates that light from the invisible infra-red part of solar radiation is completely absorbed regardless of the coat colour. However, the absorption of the visible portion of radiation is dependent on coat colour. The studies of Archarya *et al.* (1995) and others (Finch *et al.*, 1984; Helal *et al.*, 2010) have shown that

dark coats absorb more solar radiation than lighter coats. Therefore, the coat colour of animals is important for adaptability in hot-humid environment.

The dominance of dark coloured goats in hot arid regions is, however, paradoxical. Singh *et al.* (1997) showed that dark coat colour, which is preponderant in desert goats, serves as an adaptation mechanism to economize feed energy in cool period. Daramola and Adeloye (2009) hypothesized that the absorption of solar radiation by dark coloured goats maybe of significant advantage, in terms of thermoregulation, during winter months or cooler nights and that the heat absorbed at the surface of black hair may be dissipated by convection when there is adequate wind velocity.

2.6 The Role of Coat Colour in Disease Development

Mutations in the genes governing coat colour in mammals lead to different shades of coat colour, altered biological functions and disease phenotypes. Examples of diseases associated with coat colour include: white heifer disease, hearing loss, overo lethal white syndrome (OLWS), Chediak-Higashi syndrome, amyloidosis, cancer eye or ocular squamous cell carcinoma, etc.

White heifer disease, observed in Shorthorn and Belgian Blue breeds of cattle, is a developmental anomaly of the female bovine genital tract that leads to sterility (Charlier *et al.*, 1996; Hanset, 1985). Hanset (1985) suggested that the White heifer disease is caused by homozygosity of the Roan locus (*R*/R), since more than 90% of affected animals of the Belgian Blue cattle breed have the white phenotype. According to Charlier

et al. (1996), white heifer disease has a multifactorial basis with the roan locus being the principal genetic determinant, acting in conjunction with one or more auxiliary genes.

The Overo Lethal White Syndrome (OLWS) in paint horses, characterized by myenteric aganglionosis and fatal functional intestinal obstruction, is known to result from mutations in the Endothelin Receptor B (EDNRB) or endothelin 3, one of EDNRB ligands (Metallinos *et al.*, 1998; Santschi *et al.*, 1998). The developmental regulation of neural crest cells, the progenitors of melanocytes, is governed by the endothelin signaling pathway. Metallinos *et al.* (1998) and Santschi *et al.* (1998) found the substitution of lysine for isoleucine at codon 118 located within the first transmembrane domain of EDNRB, a seven transmembrane G protein coupled receptor, to be responsible for OLWS in paint horses. They found all lethal white foals to be homozygous for the Lys118 allele and all solid-coloured horses to be homozygous for the *Ile118* allele.

The incidence of an inheritable bleeding disorder, known as Chediak Higashi syndrome (CHS), has been reported in Japanese black cattle, Hereford and Brangus breeds of cattle by various researchers. Increased bleeding tendency, abnormal granules in leukocytes, decreased number of dense granules in platelets, and partial albinism are seen in affected animals (Renshaw *et al.*, 1974; Kuneida *et al.*, 1990; Yamakuchi *et al.*, 2002; Gutiérrez-Gil *et al.*, 2007). Kunieda *et al.* (1990) reported a nucleotide substitution of A to G transition in the *Lysosomal Trafficking Regulator* (*LYST)* gene also known as *CHS* gene, resulting in an amino acid substitution of histidine to arginine at codon 2015 (H2015R), in Japanese black cattle to be associated with the disease. *LYST* is known to encode a putative membrane-associated protein regulating intracellular protein trafficking

(Kuneida *et al.*, 1990; Charette and Cosson, 2007). Mutation in *LYST* may therefore cause defects in subsets of vesicles, such as melanosomes (the principal vesicle in mammalian pigmentation), lysozymes, platelet dense granules, and cytolytic granules (Kuneida *et al.*, 1990).

Hearing loss, observed in some albino mammals, is also attributed to albinism since melanin pigment is associated with the inner hearing mechanism. Congenital deafness associated with pigmentation has been reported in many mammalian species, including mink, horses, dogs, cats, etc (Henry *et al.*, 1975; Conlee *et al.*, 1986; Gottesberge, 1987; Bartels *et al.*, 2001; Famula *et al.*, 2007). For example, Famula *et al.* (2007) observed a statistically significant influence of coat colour on the incidence of deafness in Jack Russell Terrier dogs. Dogs with more white furs were found to be more likely to be deaf.

2.7 Classification of the domestic goat

Kingdom:	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Artiodactyla
Suborder	Ruminantia
Family:	Bovidae
Genus:	*Capra*
Species:	*Capra hircus*
Common Name:	domestic goat, goat

Source: Harris, 1962.

2.8 Domestication of the wild ancestors of goat

Archaeological evidence indicates that goat was one of the first animals to be domesticated by humans around 10,000 years ago at the dawn of the Neolithic period in the "Fertile Crescent" (Manceau *et al.,* 1999; MacHugh and Bradley, 2001; Joshi *et al.,* 2004). Goats played a central role in the Neolithic agricultural revolution and the spread of human civilization around the globe (MacHugh and Bradley, 2001; Joshi *et al.*, 2004).

The origin of the domestic goat remains uncertain and controversial, despite the archaeological evidence. Two hypotheses have been put forward to explain the origin of the domestic goat. One hypothesis states that at least two wild species of *Capra* contributed to the gene pool of domestic goats. Another hypothesis states that present day goats originated from the wild ancestor, *Capra aegagrus* (bezoar). Luikart *et al.* (2001) demonstrated three highly divergent goat mitochondrial DNA (mtDNA) lineages on carrying out a worldwide survey of domestic goat mtDNA diversity. From their results, lineage A was the most common in all continents, lineage B was found in the Indian subcontinent, Mongolia, and Southeast Asia, while lineage C was observed in a few samples from Mongolia, Switzerland, and Slovenia. They estimated the divergence time of the three lineages to be over 200,000 years ago. They, therefore, presumed that this ancient divergence time and the different geographical localizations of the lineages points to either multiple domestication events or introgression of additional lineages after the original domestication. Based on these and the fact that recent mtDNA studies in each of the other major farm animals (cattle, sheep, and pigs) have also revealed multiple highly

divergent lineages, they concluded that multiple maternal origin is the plausible explanation for the origin of the domestic goat.

Previous work by Manceau *et al.* (1999), however, supports the origination of the present day domestic goats from *C. aegagrus* (bezoar). Manceau *et al.* (1999) analyzed mtDNA sequences obtained from the following wild species: *C. aegagrus, C. ibex caucasica, C. cylindricornis, C. falconeri, C. i. sibirica, C. i. nubiana, C. pyrenaica*, and five breeds of the domestic goat, *C. hircus.* They found that the five haplotypes identified in the domestic goat, *C. hircus,* and the *C. aegagrus* haplotype were monophyletic (bootstrap values of 94% in the Neighbour Joining (NJ) and 77% in the Maximum Parsimony (MP) analyses). Their result is therefore consistent with the hypothesis of the domestication of *C. aegagrus* wild goats.

2.9 Nigerian Goat Breeds

According to Scherf (2000), there are about 570 breeds of domestic goats in the world, 15.6% (89) of which are found in Africa. Nigeria harbours three major breeds of goat, and these include: the West African Dwarf (WAD), the Sahel or Desert goat, and the Red Sokoto.

2.9.1 West African Dwarf goat

The West African Dwarf (WAD) goat is predominantly black in colour, although brown, gray, red and white, and sometimes combinations of these in a variety of patterns can be

seen (Adedeji *et al.,* 2006; Oseni *et al.*, 2006; Yakubu *et al.,* 2010). They are short-legged and small-sized (Figure 4) and their small size is thought to be a form of adaptation to the environment (Blench, 1999). The WAD breed, with straight nose and upright ears, is the predominant breed of the humid and sub-humid zones of Nigeria and is widely distributed across the rainforest belt of Southern Nigeria – Figure 5 (Adedeji *et al.,* 2006; Oseni *et al.,* 2006; Yakubu *et al.,* 2010). The mature body weight of WAD varies between 22-26kg (Yakubu *et al.,* 2010). Wattles (especially the bilateral), beards and horns are present in both sexes; however wattles and beards are more frequent in males (Adedeji *et al.,* 2006; Oseni *et al.,* 2006). The smooth hair type is also predominant within this breed (Adedeji *et al.,* 2006; Yakubu *et al.,* 2010). WAD goats are hardy, trypanotolerant, and possess the ability to survive, adapt, and reproduce under harsh conditions (Adedeji *et al.,* 2006; Chiejina and Behnke, 2011; Yakubu *et al.,* 2011).

Figure 4: A typical West African Dwarf (WAD) goat.

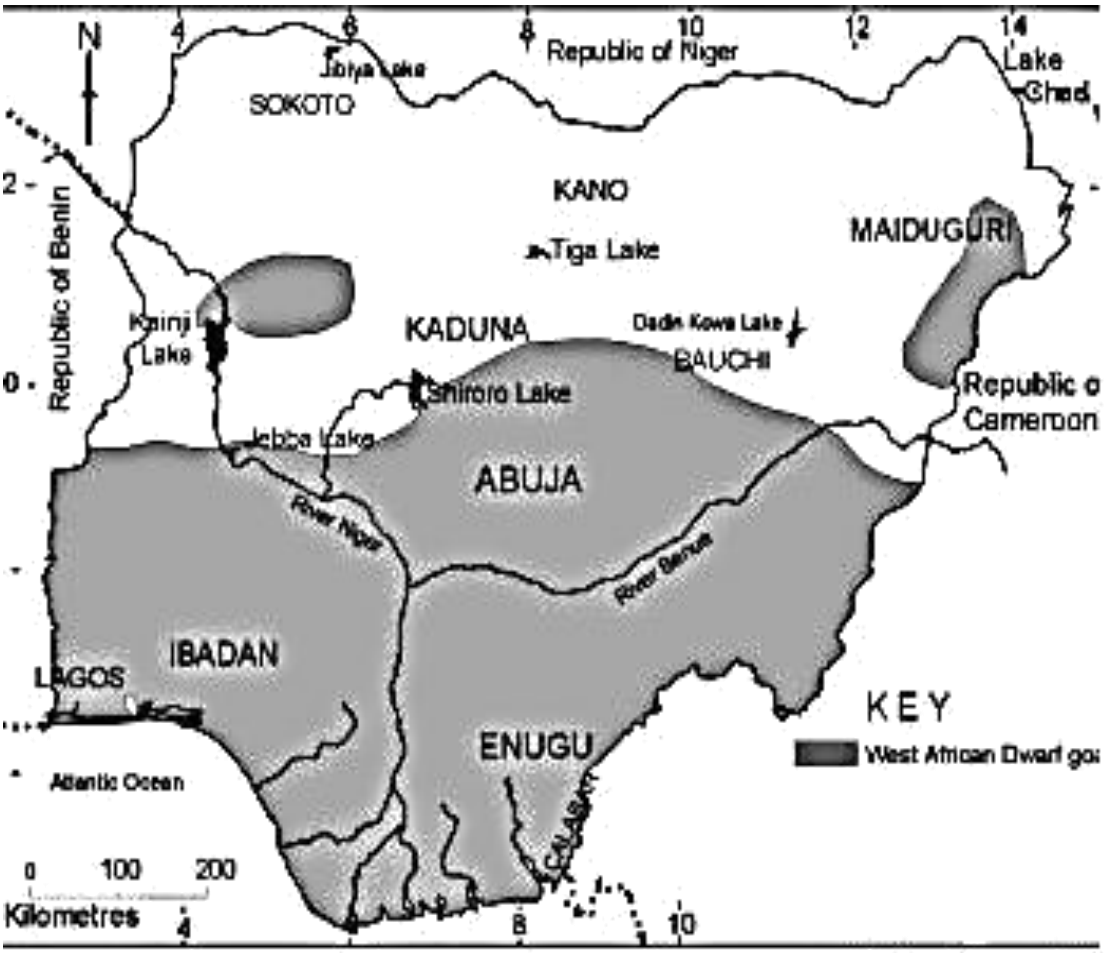

Figure 5: Map of Nigeria showing the geographical distribution of the West African Dwarf goat

Source: Blench, 1999.

2.9.2 Red Sokoto goat

The Red Sokoto, Kano Brown or Maradi goat is the most widespread and well-known breed of goat in Nigeria. It is widely distributed in the northern part of Nigeria – Figure 6 (Blench, 1999; Yakubu *et al.*, 2011). Red Sokoto goats are predominantly red in colour with mostly smooth hair type (Yakubu *et al.,* 2010). They may have black stripe with

black tail hair – Figure 7. Wattles, beards and horns are usually present in both sexes, with preponderance of wattles and beards in the males; the WAD goats however show higher incidences of wattles and beards (Yakubu *et al.,* 2010). They have fine head with prominent forehead, short and thin neck, and short ears which are usually carried horizontally (Wilson, 1991). The Red Sokoto goats have higher body height and weight compared to WAD goats (Yakubu *et al.,* 2011). The body weight of adult goats range between 28.49 and 30.80kg (Hassan and Ciroma, 1992). WAD goats, however, have larger chest girth, width of chest and depth of chest (that are related to fatness) (Attah *et al.,* 2004). Red Sokoto goats are known for their high-quality skin which is used for making leather products (Blench, 1999; Yakubu *et al.*, 2011).

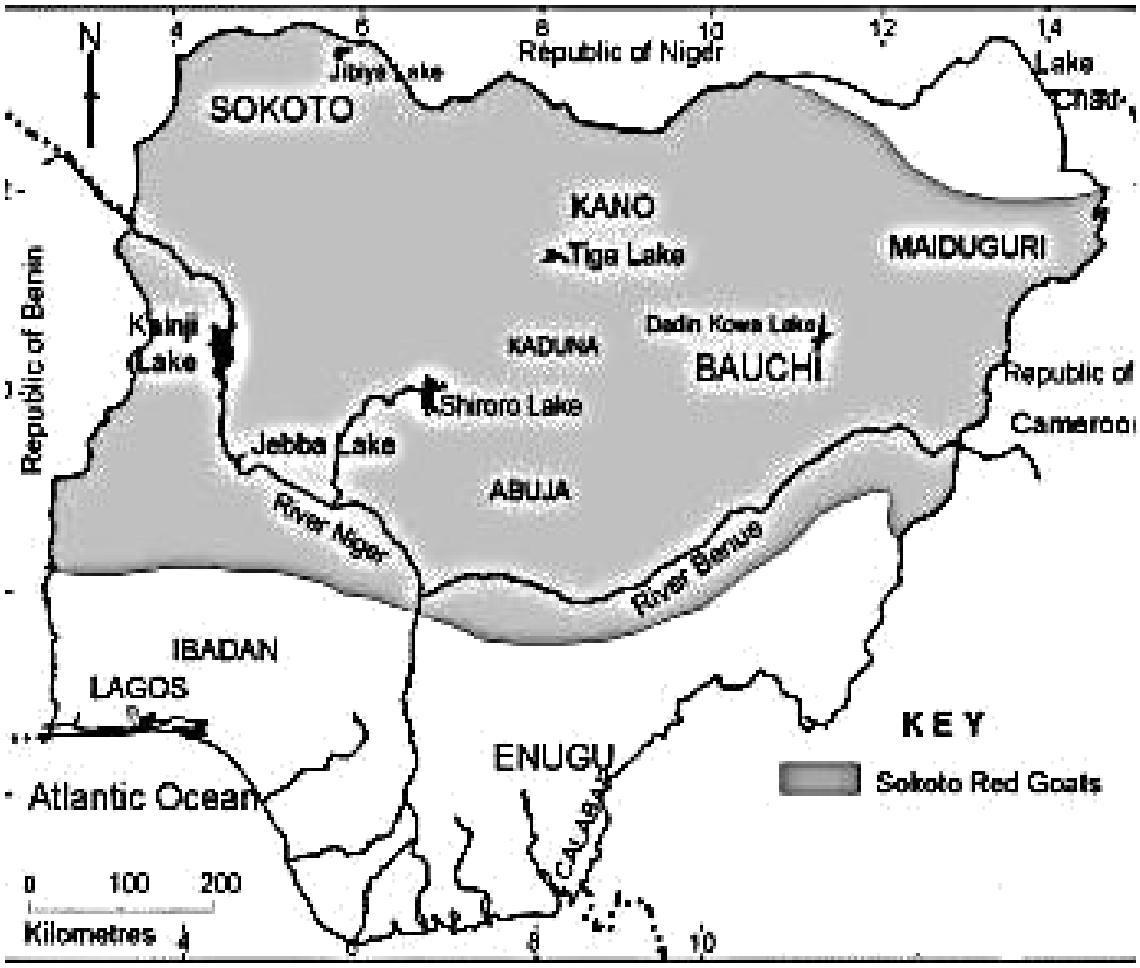

Figure 6: Map of Nigeria showing the geographical distribution of the Red Sokoto goat

Source: Blench, 1999.

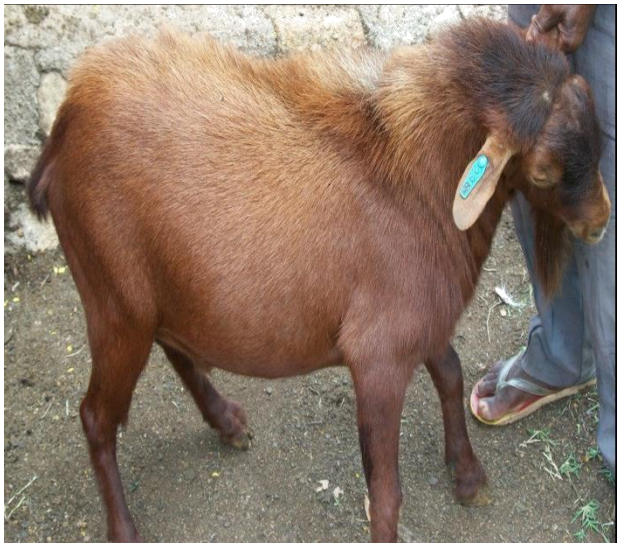

Figure 7: A typical Red Sokoto goat.

2.9.3 Sahel or Desert goat

The Sahelian or Desert goat is found along the northern border of Nigeria (Figure 8), particularly in Borno, where it is often known as 'Balami' or Borno white. The coat is mostly white or dappled with short, fine hair, the ears are floppy and pendulous or semi-pendulous and the legs are notably longer than the other breeds – Figure 9. They usually have narrow body, shallow chest, and long spindly legs. Both sexes are horned and most have wattles. The average mature weights of male and female Sahel goats are 38 and 33kg, respectively (Mohammed and Amin, 1996; Blench, 1999).

Figure 8: Map of Nigeria showing the geographical distribution of the Sahel goat

Source: Blench, 1999.

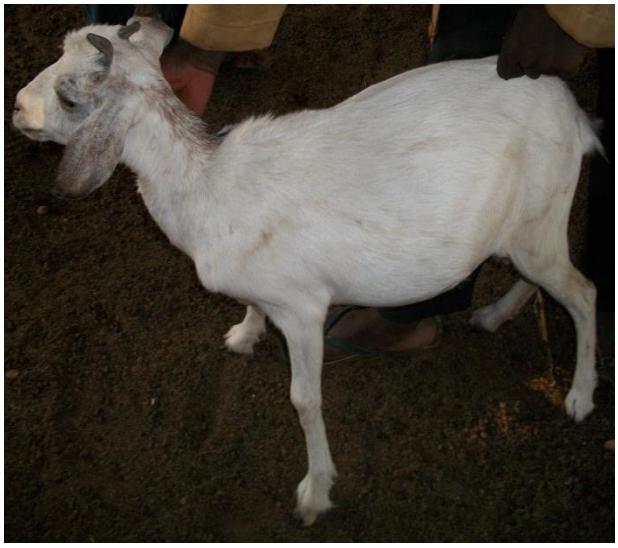

Figure 9: A typical Sahel goat.

2.10 Studied American Goat Breeds

2.10.1 Alpine goat

The colour pattern of the Alpine breed ranges from pure white through shades of fawn, gray, brow, black, red, bluff, piebald or various shadings or combinations of these colours. The black and white Alpine were used in this study. Both sexes of the Alpine breed are generally short haired, but bucks usually have a roach of long hair along the spine. The beard of males is also quite pronounced. The ears of Alpine goats are usually medium size, fine textured and preferably erect. Average withers height and weight of matured female Alpine goats are 76.2cm and 61.23kg, respectively. Average withers height and weight of matured male Alpine goats are 101.6cm and 77.11kg, respectively.

Female Alpine goats are excellent milkers. They are hardy animals that thrive in any climate while maintaining good health and excellent production.

Figure 10: An Alpine goat used in this study

2.10.2 Spanish goat

The Spanish goat can be any colour but the brown and white Spanish goats were used in this study. The Spanish goats range in weight from 22.68 to 90.72kg, with the largest animals representing strains that have been selected over many decades for meat production. Spanish goats are usually horned and the horns on bucks may be large and twisted. The ears are large, and are held horizontally and forward, very close to the head. They may have long ears with distinct ear carriage and straight or concave face. The Spanish goat is unique to the United States. They were brought from Spain to the Caribbean Islands in the 1500s and from there to the areas that became the United States

and Mexico. These foundation stocks were an undifferentiated Mediterranean type of goat that was common in Spain at that time but no longer exists. The majority of Spanish goats in the United States were crossed with imported breeds, especially Boer goats (Cornell University Cooperative Extension, 2010). Boer-Spanish crosses are known to perform well.

Figure 11: A Spanish goat used in this study

2.10.3 Saanen goat

The Saanen goat is a dairy goat. It originated in Switzerland, in the Saanen Valley. Saanen goats are medium to large in size, weighing approximately 65kg. Saanens are white or light cream in colour. The Saanen goats used in this study are white in colour. The ears of Saanen goats are usually erect and pointing forward.

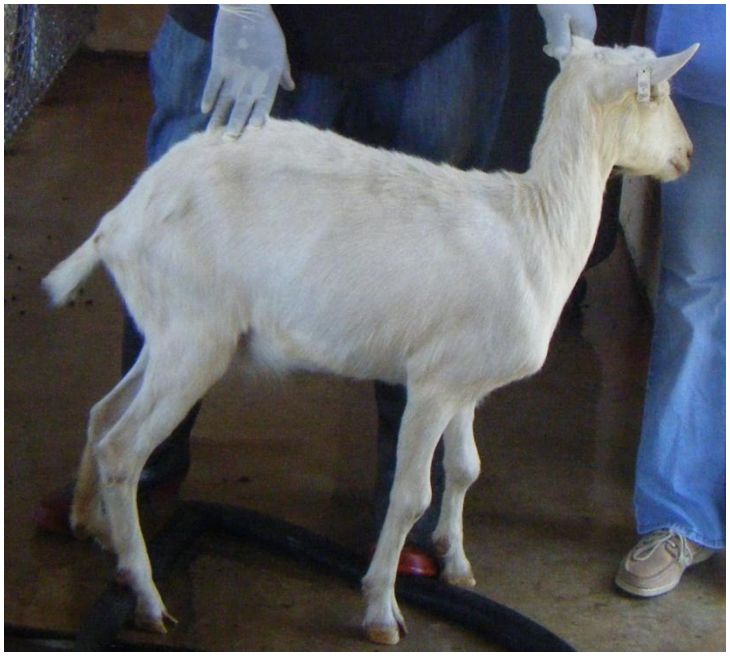

Figure 12: A Saanen goat used in this study

3.0 MATERIALS AND METHODS

3.1 Animal Sampling and DNA Isolation

Four hundred and nine (381) goats of the three major breeds in Nigeria; West African Dwarf (n=137), Red Sokoto (n=187) and Sahel (n=57); were sampled, from farms and markets across different states in Nigeria, according to the geographical distribution of the breeds published by Blench (1999). Animals were not sampled from the South East as a large number of West African Dwarf goats, which is the breed common to the South East, were already sampled from the South West. Tables 1 and 2 show the sampling details of goats used in this study and the sites of sample collection are shown in Figure 13. In addition, 23 Alpine, 13 Spanish and 9 Saanen goats were sampled from Fort Valley State University, Georgia, United States of America. Animals were sampled from the United States as the opportunity was available since most of the bench work was carried out at Cornell University, Ithaca, New York. Goats are rarely reared at Cornell University, as a result animals were sampled from Fort Valley State University where collaboration was made. Pictures or coat colour descriptions were taken for all animals.

Seven milliliters (7ml) of blood was collected from each goat by jugular venipuncture into heparinized tubes and stored at 4^{O}C before they were transferred to the laboratory for DNA isolation. Genomic DNA from collected blood samples were isolated using ZymoBead^{TM} Genomic DNA kit (Zymo Research Corp. Irvine, CA, USA) as follows:

- ZymoBead™ slurry was fully resuspended by swirling using a vortexer. In a 1.5 ml tube, 200 µl of Genomic Lysis Buffer was added to 50 µl of blood,

after which 10 µl of ZymoBeads™ was added. The mixture was mixed by inversion, and then incubated at room temperature for 5 minutes. The tube was centrifuged at 1,500*g* for 1 minute. The supernatant was thereafter carefully removed without disturbing the bead pellet.

- The pellet was resuspended by adding 200 µl of Genomic Lysis Buffer and pipetting up and down. The tube was thereafter centrifuged at 1,500*g* for 1 minute and the supernatant was discarded.
- The pellet was resuspended by adding 200 µl of DNA Pre-Wash Buffer to the ZymoBeads™. The suspension was transferred to a new tube, and then centrifuged at 1,500*g* for 1 minute. The supernatant was thereafter discarded.
- The pellet was again resuspended by adding 500 µl of g-DNA Wash Buffer to the ZymoBeads™ and then centrifuged at 1,500*g* for 1 minute. The supernatant was again discarded. The tube was recentrifuged briefly and residual wash buffer was removed.
- The pellet was resuspended by adding 35 µl of Elution Buffer and pipetting up and down. The tube was then centrifuged at 10,000*g* for 1 minute
- The supernatant which contained the purified DNA was collected and stored at -20ºC for later use.

Quantification of DNA yield and assessment of quality were done using a Nanodrop ND-100 UV/Vis Spectrophotometer (Nanodrop Technologies, Inc., DE, USA).

Table 1: Sampling details of Nigerian goats used in this study (n = number of animals)

Location	Code	Latitude	Longitude	n
Nassarawa	A	N08.38.790	E008.33.542	6
Nassarawa	B	N08.38.831	E008.33.504	8
Nassarawa	C	N08.33.511	E008.33.076	6
Jos	D	N010.01.399	E008.51.043	36
Bauchi	E	N010.17.044	E009.47.644	44
Maiduguri	F	N011.47.553	E013.12.482	43
Maiduguri	G	N011.51.574	E013.10.531	20
Kano	H	N011.58.590	E008.25.815	14
Zaria	I	N011.47.764	E008.38.728	28
Ipokia (Ogun)	J	N06.30.434	E002.43.124	50
Abeokuta	K	N06.27.951	E002.43.153	27
Abeokuta	L	N07.10.184	E003.21.093	16
Abeokuta	M	N07.08.408	E003.21.068	16
Kebbi	N	N11.30.951	E013.43.153	20
Zaria	O	N11.07.951	E008.38.283	28
Sokoto	P	N13.05	E5.23	5
Sokoto	Q	N13.25	E4.67	5
Sokoto	R	N12.62	E4.78	4
Sokoto	S	N11.4	E4.12	5
Total				**381**

Table 2: Sample sizes by coat colour, age group, breed and sex for the goats studied

Coat colour	Age (yrs)	West African Dwarf			Red Sokoto			Sahel		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
Black	1-2	4	8	12	1	0	1	2	1	3
	2-3	0	10	10	0	1	1	0	0	0
	3-4	0	3	3	0	0	0	0	0	0
	Total	4	21	25	1	1	2	2	1	3
Brown	1-2	6	12	18	1	0	1	0	1	1
	2-3	0	12	12	0	1	1	0	0	0
	3-4	0	1	1	2	2	4	1	3	4
	Total	6	25	31	3	3	6	1	4	5
Red	1-2	3	2	5	22	46	68	0	1	1
	2-3	0	3	3	11	44	55	2	2	4
	3-4	0	4	4	10	40	50	1	2	3
	Total	3	9	12	43	130	173	3	5	8
White	1-2	18	26	44	1	0	1	4	5	9
	2-3	7	15	22	0	2	2	11	5	16
	3-4	0	3	3	2	1	3	7	9	16
	Total	25	44	69	3	3	6	22	19	41
Total		38	96	137	34	129	187	27	28	57

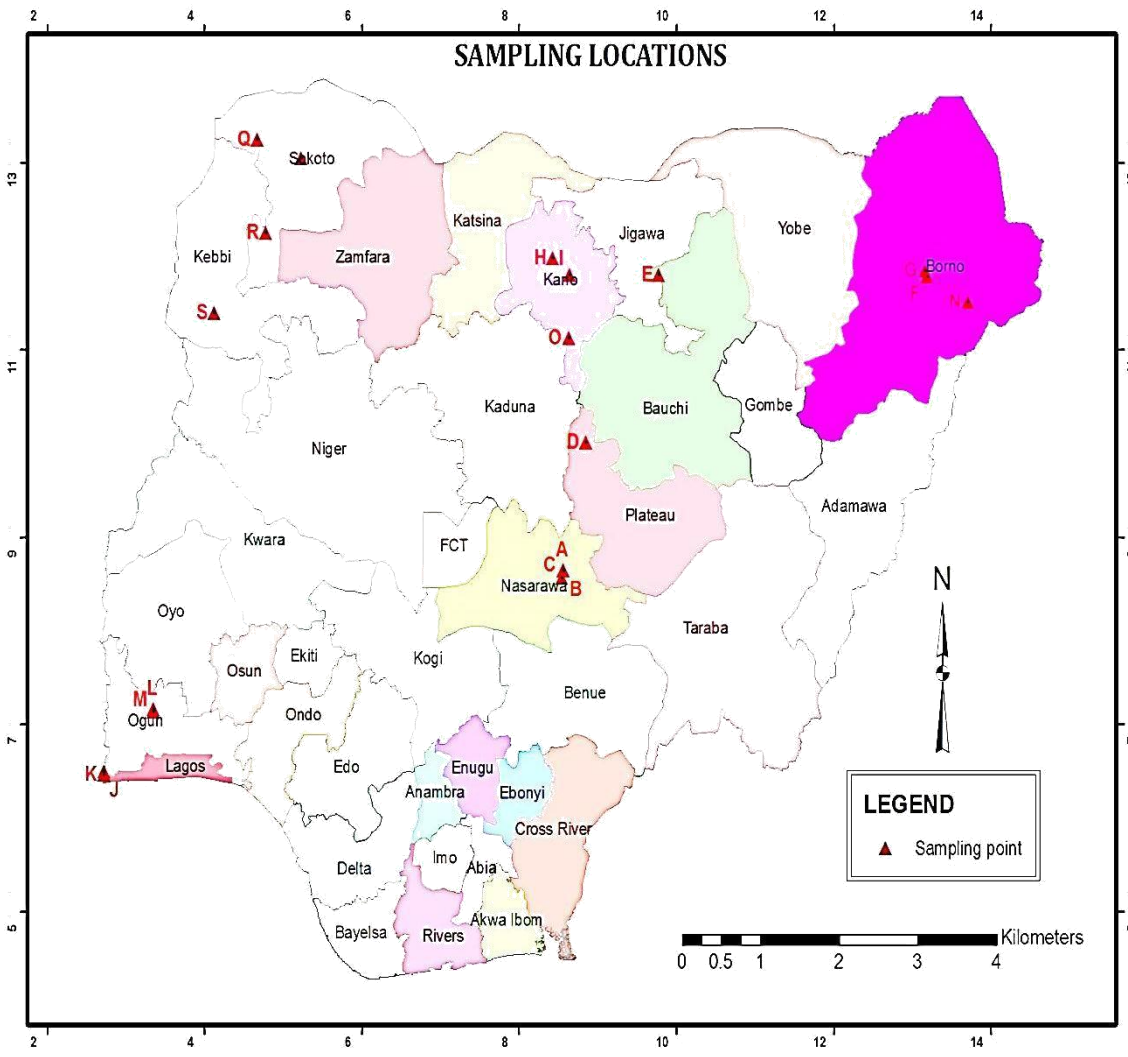

Figure 13: Map of Nigeria showing sites of sampling collection (▲ indicates sampling location). The sampling sites are designated A to S (see Table 1).

3.2 Morphological Traits Measurement of Nigerian Indigenous Goats

3.2.1 Morphological Traits Measurement

The following measurements were taken on each sampled Nigerian goat, following standard procedure (Yakubu and Akinyemi 2010; Yakubu *et al.*, 2010) and anatomical reference points as shown in Figure 14:

Body weight (BW): This was taken in kilogram (kg) using a hanging scale. The animals were turned on their back in a Hessian bag and the weight was taken as the difference between the final weight and the weight of the bag.

Body length (BL): Body length was measured in centimeter (cm), using a tape-rule, as the distance from the occipital protuberance to the pin bone.

Height at wither (HW): A flat platform was used upon which the animals were placed. The height at wither was measured in centimeter (cm) as the distance from the surface of the platform to the withers using a measuring stick.

Heart girth (HG): The heart girth was measured in centimeter (cm) by taking the measurement of the circumference of the chest with a tape rule.

Rump height (RH): The rump height was measured in centimeter (cm) as the distance from the surface of the platform to the rump using a measuring stick.

Ear length (EL): This was measured in centimeter (cm) as the distance from the base of the ear to its tip using a tape rule.

Fore cannon bone length (FCL): This was measured in centimeter (cm) as the length of the lower part of the leg extending from the hock to the fetlock using a measuring stick.

Tail length (TL): This was measured in centimeter (cm) as the distance from the base of the tail to the end of the coccygeal vertebrae with a tape rule.

Chest depth (CD): This was measured in centimeter (cm) as the dorsal–ventral distance between the most dorsal point of the withers and the ventral surface of the sternum using a measuring stick.

Rump width (RW): This was measured in centimeter (cm) as the distance between the two *tuber coxae* with a measuring stick.

Each animal was identified by breed, sex and estimated age class based on dentition (Gerald, 1994; Peacock, 1996) using permanent teeth eruption. Only animals that conformed to the classification descriptors of each breed of goat were sampled. Care was also taken to avoid measuring sick, pregnant, crossbreeds and closely related animals. These were done with the help of the following Animal Breeders: Mr. Abdulmojeed Yakubu, Mr. Mathew Whetto and Mr. Samuel Amusan.

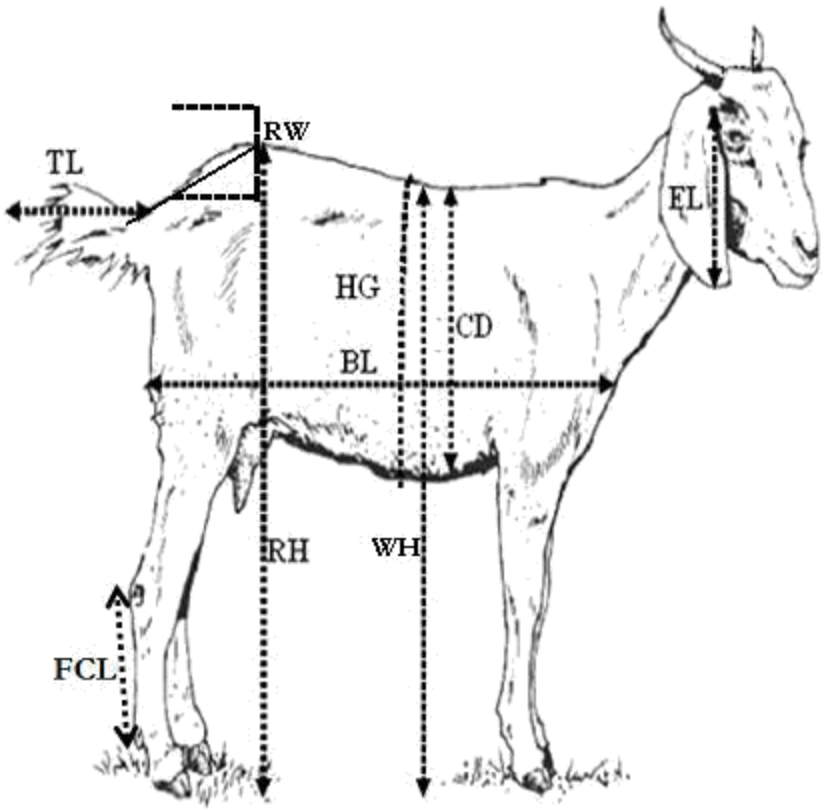

Figure 14: Body measurements taken. BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; FCL = Fore cannon bone length; EL = Ear length; RW = Rump Width

3.2.2 Statistical Analysis

All data were checked for skewness using SPSS (2010) statistical package UNIVARIATE procedure and found to conform to normal distribution. Mean and standard errors were computed for each body measurement. The General Linear Model (GLM) procedure of the same software package was used to analyze the data fitting breed, age, sex and coat colour as main effects in the model. After the removal of non-significant interactions, the following model was employed:

$Y_{ijklm} = \mu + C_i + B_j + S_k + A_l + (CB)_{ij} + (CSA)_{ikl} +$

e_{ijklm} Where Y_{ijklm} = individual observation

μ = Population mean

C_i = Effect of the i^{th} coat colour

B_j = Effect of the j^{th} breed

S_k = Effect of the k^{th} sex

A_l = Effect of the l^{th} age

$(CB)_{ij}$ = Effect of the interaction of the i^{th} coat colour and j^{th} breed

$(CSA)_{ikl}$ = Effect of the interaction of the i^{th} coat colour, k^{th} sex and l^{th} age

e_{ijklm} = Random residual error normally and independently distributed with zero mean and common variance.

3.3 DNA Polymorphisms and Haplotype Patterns of the *Melanocortin 1 Receptor* (*MC1R)* and *Agouti Signaling Protein* (*ASIP*) Genes

3.3.1 Animals and DNA samples

For the studies on *MC1R* gene, genomic DNA isolated from blood samples collected from 105 of the 381 sampled Nigerian goats, comprising of 32 West African Dwarf goats, 39 Red Sokoto goats and 34 Sahel goats, as well as 40 of the 45 sampled American goats, comprising of 20 Alpine goats, 13 Spanish goats and 7 Saanen goats were used in this study.

Genomic DNA isolated from blood samples collected from the following goat breeds: 62 West African Dwarf, 92 Red Sokoto, 32 Sahel, 15 Alpine, 7 Spanish and 7 Saanen were used for the studies on *ASIP* gene.

3.3.2 Isolation and Sequencing of *MC1R* and *ASIP* Gene fragments

The following primers, which were designed with Primer 3 (Whitehead Institute for Biomedical Research, Cambridge, MA) from the caprine *MC1R* sequence with EMBL accession number FM212940 containing the whole coding region and parts of the 5'- and 3'-untranslated regions, were used for *MC1R* amplification and sequencing:

F: 5'-CGATGCCTGCACTCGGCTCC-3'

R: 5'-GCCCTTGAGGCCAAAGCCCT-3'

According to the report of Fontanesi *et al.* (2009b), the following pair or primers was synthesized to amplify a 352 bp product spanning part of exon 2 and the whole intron 2 of the *ASIP* gene:

F: 5'- GGTCAGAGTACCAGCCCAAA-3'

R: 5'- GTATCGGCTTGGGGAGTGTT-3'

PCR amplifications were carried out in a C1000^{TM} Thermal Cycler (Bio-Rad, USA) with a total reaction volume of 20µL containing 20-80ng DNA, 10pmol of each primer in 10µL Syd Lab PCR Premix (Syd Labs, Inc., Malden, USA) containing Taq DNA polymerase, dNTPs, $MgCl_2$, reaction buffer, PCR stabilizer and enhancer at optimal concentrations. The PCR cycling conditions are as follows: denaturation at 95^{o}C for 4 min, followed by 35 amplification cycles of denaturation at 94^{o}C for 30S, annealing at 66.2^{o}C for the *MC1R* gene and 56^{o}C for the *ASIP* gene for 30S, extension at 72^{o}C for 1 min, followed by an extended elongation at 72^{o}C for 10 min. PCR products were detected on 1.5% agarose gel including 0.5µg/ml of ethidium bromide, photographed under UV light and scored using GeneMate Quanti-Marker 100 bp DNA ladder (BioExpress, UT, USA). Sequencing of the amplified fragments was carried out using the same PCR primer with the Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase.

3.3.3 SNP Identification and Genotyping

Sequence bases were called using Phred and assembled using Phrap as implemented in the Codoncode aligner 3.7.1 software package http://www.codoncode.com/aligner. Single nucleotide polymorphisms were identified with the aid of CodonCode Aligner software and DnaSP version 5.10.01. Nomenclature of the detected SNPs was obtained following the nomenclature for the description of sequence variation (http://www.hgvs.org/mutnomen/) using the sequence with EMBL accession number FM212940.1 as reference for the *MC1R* gene and AM746057.2 as reference for the *ASIP* gene. Amino acid sequences of the amplified fragments were predicted using the translator program (http://www.fr33.net/translator.php). Genotyping was done by analyzing and comparing the obtained sequence electropherograms.

3.3.4 Sequence Analysis and Statistics

Indices of sequence variation and haplotype structure were calculated using the DnaSP version 5.10.01 (Rozas, 2003) including number of polymorphic sites, nucleotide diversity (π), number of haplotypes and haplotype diversity. The hypothesis of neutral polymorphisms was also tested using Tajima's D test (Tajima, 1989). Non-significant value of Tajima's D indicates no evidence for evolutionary selection.

In silico functional analysis of missense mutations was obtained using PANTHER (Protein Analysis THrough Evolutionary Relationships) whose predictions have been experimentally validated (Thomas *et al.,* 2003; Brunham *et al.,* 2005). PANTHER

estimates the likelihood of a particular non-synonymous (amino acid changing) coding SNP to cause a functional impact on the protein. It calculates the substitution Position-Specific Evolutionary Conservation (subPSEC) score based on an alignment of evolutionarily related proteins (Thomas *et al.,* 2003; Thomas *et al*., 2006). The probability that a given variant will cause a deleterious effect on protein function is estimated by $P_{deleterious}$, such that a subPSEC score of -3 corresponds to a $P_{deleterious}$ of 0.5 (Brunham *et al.,* 2005). The subPSEC score is the negative logarithm of the probability ratio of the wild-type and mutant amino acids at a particular position. PANTHER subPSEC scores are continuous values from 0 (neutral) to about -10 (most likely to be deleterious).

Haplotypes were inferred using DnaSP version 5.10.01. A median-joining network (Bandelt *et al.,* 1999) for these haplotypes was constructed using Network version 4.510 (http://www.fluxus-engineering.com). Peptide structure of *MC1R* was predicted using tmap program (http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::tmap) and the secondary structure of the amino acid sequence was drawn using the transmembrane protein display software, TOPO2 (topology) (www.sacs.ucsf.edu/TOPO). TOPO2 is a software for creating transmembrane protein 2D topology images. Alignment of the amino acid sequences of goat with those of some other species was also done using ClustalX.

3.4 Variation of *g.469*G>C in the Melanophilin (*MLPH*) Gene Intron 2 in Nigerian Goat Breeds

3.4.1 Animals and DNA samples

In this study, blood samples of 266 goats belonging to the three Nigerian indigenous goat breeds; 80 West African Dwarf, 135 Red Sokoto and 51 Sahel, with detailed coat colour record, were taken from those of the 352 Nigerian sampled goats. Genomic DNA from collected blood samples was isolated using ZymoBead^{TM} Genomic DNA kit following the manufacturer's instruction as described in section 3.1.

3.4.2 Isolation and Sequencing of *Melanophilin* Gene fragments

According to the report of Feng *et al.* (2009), the following pair of primers was synthesized to amplify a 632bp fragment, spanning intron 1, exon 2 and part of intron 2:

F: 5'-CGTGGGTTCCCTTATTTTGAC-3'

R: 5'- ATCCTGGCTTCTGGGTGTTG-3'

PCR amplifications were carried out in a C1000^{TM} Thermal Cycler (Bio-Rad, USA) with a total reaction volume of 20µL containing 20-80ng DNA, 10pmol of each primer in AccuPower^{TM} PCR Premix (Bioneer Corporation, USA). The PCR cycling conditions are as follows: denaturation at 95^{o}C for 4 min, followed by 35 amplification cycles of denaturation at 94^{o}C for 30 s, annealing at 56^{o}C for 30S, extension at 72^{o}C for 1 min, followed by an extended elongation at 72^{o}C for 10 min. PCR products were detected on

1.5% agarose gel including 0.5µg/ml of ethidium bromide, photographed under UV light, and scored using GeneMate Quanti-Marker 100 bp DNA ladder (BioExpress, UT, USA). Sequencing of the amplified fragments was carried out using the same PCR primer with the Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase.

3.4.3 Genotyping of goat *MLPH* by PCR-RFLP

In order to identify SNPs of melanophilin gene in Nigerian goats, sequences derived from 20 animals were aligned using ClustalX software program (http://www.ebi.ac.uk/Tools/clustalx/). The transversion *469C>G*, according to EU316218, was detected. This SNP was analyzed by PCR-RFLP in a total of 266 goats. *Hinp1*I (Fermentas Life Sciences, USA), recognizing the palindromic tetranucleotide sequence G↓CGC, was used for restriction enzyme digestion according to nebcutter V2.0. Aliquots of 15µL PCR products, including 2µL 10X buffer with Bovine serum Albumin (BSA), were digested with 1µL (10U) *Hinp1*I for 16 h at 37^{o}C. Thermal inactivation of the restriction enzyme was thereafter done by incubating at 65^{o}C for 20 min. The digested products were detected by electrophoresis on 2.0% agarose gel including 0.5µg/mL of ethidium bromide.

3.4.4 Statistical analysis

Genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibrium test were performed using PopGene32 software version 1.32. The genetic diversity of each breed and population and genetic differentiation among different breeds were estimated using the same software.

3.5 Association between identified mutations and some body traits in Nigerian goats

Only mutations identified at the *MC1R* locus were analyzed for association between genotypes and body traits as they were the only ones observed to have some possible relationship with coat colour.

Genotypes of animals were determined by analyzing and comparing the obtained sequence electropherograms as discussed in section 3.3.3. All the body traits taken, as discussed in section 3.2.1, were included in this analysis. Data were checked for skewness using SPSS (2010) statistical package UNIVARIATE procedure and found to conform to normal distribution. Mean and standard errors were computed for each body measurement. The General Linear Model (GLM) procedure of the same software package was used to analyze the data fitting breed, age, sex and genotype as main effects in the model. The following model was generally employed:

$Y_{ijkm} = \mu + G_i + B_j + S_k + (GB)_{ij} + (GS)_{ik} + (BS)_{jk} + (GBS)_{ijk} +$

e_{ijkm} Where Y_{ijkm} = individual observation

μ = Population mean

G_i = Effect of the i^{th} genotype

B_j = Effect of the j^{th} breed

S_k = Effect of the k^{th} sex

$(GB)_{ij}$ = Effect of the interaction of the i^{th} genotype and j^{th} breed $(GS)_{ik}$ = Effect of the interaction of i^{th} genotype and k^{th} sex $(BS)_{jk}$ = Effect of the interaction of j^{th} breed and k^{th} sex

$(GBS)_{ijk}$ = Effect of the interaction of the i^{th} genotype, j^{th} breed and k^{th} sex

e_{ijkm} = Random residual error normally and independently distributed with zero mean and common variance.

4.0 RESULTS

4.1 Effect of Coat Colour on Some Morphological Traits of Nigerian Indigenous Goats

This study looked at the effect of coat colour on some morphometric characters. In addition, comparisons were made between breeds, age groups and sexes to provide evidence of the effect of these factors on morphological traits. A summary description of the analyses of variance is shown in Table 3 and the least square means of coat colour, breed, age and sex for the morphological traits studied are presented in Tables 4, 5, 6 and 7, respectively. From Table 3, coat colour was found to significantly affect body weight ($P<0.01$), body length ($P<0.05$) and heart girth ($P<0.01$). All the measurements except for rump width and ear length were significantly ($P<0.001$) affected by breed type. Sex influenced ($P<0.01$) only heart girth while age influenced ($P<0.01$) all but tail length.

The interaction of coat colour and breed significantly ($P<0.01$) influenced withers height, rump height and fore cannon bone length. Only body length and heart girth were significantly ($P<0.05$) affected by the interaction of coat colour, sex and age. The effect of breed seems to dominate that of coat colour when looking at the effect of the interaction of coat colour and breed on morphological traits as none of the factors influenced by coat colour alone seems to be influenced by the interaction of these factors. Coat colour seems to strongly determine body length, which indicates size, and heart

girth, that indicates fat and muscle deposition, as it was found to significantly influence these body traits singly and in combination with sex and age.

Table 3: Analysis of variance for morphological traits in the three Nigerian goat breeds

Source of Variation	Mean squares and level of significance									
	DF	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
Coat colour	3	123.24^{b}	232.60^{c}	4.43^{d}	36.73^{d}	2.72^{d}	158.56^{b}	2.86^{d}	0.94^{d}	2.04^{d}	1.68^{d}
Breed	2	176.29^{a}	870.64^{a}	21.38^{a}	1500.27^{a}	1177.76^{a}	406.68^{a}	131.47^{a}	5.94^{d}	98.85^{a}	2.14^{d}
Sex	1	93.60^{d}	0.62^{d}	0.01^{d}	1.31^{d}	46.72^{d}	310.48^{b}	4.36^{d}	0.03^{d}	1.48^{d}	0.04^{d}
Age	2	343.73^{a}	845.25^{a}	2.48^{d}	241.01^{a}	656.39^{a}	594.98^{a}	80.73^{a}	34.12^{a}	11.30^{b}	11.95^{a}
Coat colour x Breed	6	15.18^{d}	124.84^{d}	4.58^{d}	85.19^{b}	109.76^{b}	57.03^{d}	9.05^{d}	1.98^{d}	6.90^{b}	3.00^{d}
Coat colour x Sex x Age	1	74.71^{d}	442.72^{c}	0.34^{d}	12.69^{d}	41.67^{d}	245.66^{c}	1.97^{d}	6.91^{d}	2.11^{d}	5.65^{d}
Residual	303	29.44	81.57	2.37	26.71	39.88	49.31	10.90	2.57	2.28	1.84

DF = Degree of freedom; BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

^{a,b,c},Significant at P<0.001, P<0.01 and P<0.05, respectively; ^{d} Not significant

4.1.1 Effect of coat colour on morphological traits

Table 4 summarizes the least square means for morphological traits as affected by coat colour and Appendix 1 gives the pairwise comparisons between coat colours for the body measurements. Overall, the red animals had the highest values for all the traits analyzed except for the fore cannon bone length and the ear length (23.695kg for BW, 81.351cm for BL, 11.753cm for TL, 53.444cm for WH, 54.581cm for RH, 66.653cm for HG, 25.614cm for CD, and 10.981cm for RW). The order for the morphological traits was usually red, white, brown and black, i.e. the red animals usually had the highest measurements followed by the white, brown and black animals. Brown animals, however, had higher body length and rump width (76.677cm for BL and 10.724cm for RW) than white animals (74.516cm for BL and 10.615cm for RW). The white goats had the longest fore cannon bone and ear (12.593cm for FCL and 11.271cm for EL).

Except for the fore cannon bone length, there was no significant difference between the black and the brown goats for body weight and body dimensions studied. The white goats were also not significantly different from the brown goats for the studied morphological traits. The red goats were found to significantly differ from the black goats for all morphological measurements except for the tail length and ear length. The white goats significantly differed from the black goats for body weight, withers height, rump height and fore cannon bone length. Red goats also differed significantly from brown goats for body weight, withers height and heart girth. The red and white goats were also found to significantly differ for body weight, body length and heart girth.

Table 4: Least square means and their standard errors (LSmean±S.E.) for morphological traits as affected by coat colour

Coat colour	Morphological measurements									
	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
Black	16.691±1.416	73.809±2.358	11.451±0.402	47.017±1.349	49.232±1.648	60.177±1.833	23.091±0.862	9.807±0.418	11.014±0.394	10.719±0.354
Brown	18.487±1.274	76.677±2.120	10.624±0.362	49.507±1.213	53.435±1.482	61.030±1.648	24.583±0.775	10.724±0.376	12.116±0.354	10.866±0.318
Red	23.695±0.842	81.351±1.402	11.753±0.239	53.444±0.802	54.581±0.980	66.653±1.090	25.614±0.512	10.908±0.249	12.224±0.234	11.075±0.210
White	20.584±0.785	74.516±1.306	11.550±0.223	52.210±0.747	53.973±0.913	61.260±1.016	24.598±0.478	10.615±0.232	12.593±0.218	11.271±0.196

BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

4.1.2 Effect of breed on morphological traits

Table 5 summarizes the overall body measurements across breeds and Appendix 2 gives the pairwise comparisons between breeds for the body measurements. The body weight of Sahel (23.248kg) was significantly ($P<0.05$) higher than those of Red Sokoto (20.389kg) and West African Dwarf (17.815kg). Generally the body dimensions for Sahel (81.308cm for BL, 12.067cm for TL, 56.888cm for WH, 58.448cm for RH, 67.328cm for HG, 26.512cm for CD, 11.070cm for RW, 13.566cm for FCL and 11.339cm for EL) were significantly ($P<0.05$) greater than those for West African Dwarf (70.106cm for BL, 10.422cm for TL, 42.527cm for WH, 45.016cm for RH, 59.592cm for HG, 22.014cm for CD, 10.014cm for RW, 9.935cm for FCL and 10.609cm for EL). The Red Sokoto and Sahel goats did not, however, differ significantly ($P>0.05$) in body dimensions except for heart girth. Sahel goats were found to have greater heart girth than Red Sokoto goats (67.328cm for Sahel and 61.321cm for Red Sokoto). Although the differences between Red Sokoto and Sahel were not statistically significant for most of the body dimensions, generally the body measurements for Sahel goats were slightly or marginally higher than those for Red Sokoto goats. Red Sokoto had the following measurements: 79.950cm for BL, 11.736cm for TL, 54.523cm for WH, 56.845cm for RH, 25.618cm for CD, 10.744cm for RW, 12.981cm for FCL and 11.169cm for EL). Red Sokoto and West African Dwarf goats differed significantly for body weight and body dimensions, with Red Sokoto having the higher measurements, except for heart girth and ear length.

Table 5: Least square means and their standard errors (LSmean±S.E.) for morphological traits as affected by breed

Breed	Morphological measurements									
	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
WAD	17.815±0.658	70.106±1.095	10.422±0.187	42.527±0.627	45.016±0.766	59.592±0.851	22.014±0.400	10.014±0.194	9.935±0.183	10.609±0.164
RS	20.389±0.989	79.950±1.646	11.736±0.281	54.523±0.942	56.845±1.151	61.321±1.280	25.618±0.602	10.744±0.292	12.981±0.275	11.169±0. 247
SH	23.248±0.983	81.308±1.636	12.067±0.279	56.888±0.936	58.448±1.144	67.328±1.272	26.512±0.598	11.070±0.290	13.566±0.274	11.339±0.245

BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length WAD = West African Dwarf; RS = Red Sokoto; SH = Sahel

4.1.3 Effect of age on morphological traits

The least square means for the morphological traits as affected by age are shown in Table 6 and Appendix 3 gives the pairwise comparisons between age groups for the body measurements. Age was found to strongly influence ($P<0.05$) all the morphological traits studied except tail and ear lengths. A consistent increase in the morphological measurements was observed as the age of the animals increased. Tail length did not differ significantly ($P>0.05$) among the age groups whereas ear length differed significantly ($P<0.05$) between ages 1-2 (10.562cm) and 3-4 years (11.752cm). Although, the differences among the age groups were not statistically significant for tail and ear lengths, the measurements were slightly or marginally higher as the age of the animals increased.

4.1.4 Effect of sex on morphological traits

Table 7 shows the least square means for the studied morphological traits as affected by sex and Appendix 4 gives the pairwise comparisons between sexes for the body measurements. Body weight (19.155kg for male and 21.309kg for female) tail length (11.739cm for male and 11.147 for female), heart girth (59.976cm for male and 64.549cm for female) and fore cannon bone length (12.572cm for male and 11.800cm for female) significantly ($P<0.05$) differed between the sexes. In this study, body weight and heart girth were higher in females than males while tail and fore cannon bone lengths were higher in males than females. Although body length did not differ significantly between the sexes in this study, females were found to have higher body length than males (75.548cm for male and 77.965cm for female). Withers height (51.585cm for male

and 50.822cm for female) and rump height (54.513cm for male and 52.404cm for female) were, however, found to be greater in males than females in this study.

Table 6: Least square means and their standard errors (LSmean±S.E.) for morphological traits as affected by age

Age	Morphological measurements									
(years)	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
1-2	17.208±0.839	71.404±1.396	11.155±0.238	47.472±0.799	48.392±0.976	58.632±1.086	22.823±0.511	9.467±0.248	11.238±0.233	10.562±0.209
2-3	19.985±0.849	76.971±1.414	11.262±0.241	51.589±0.809	53.468±0.989	62.658±1.099	24.621±0.517	10.638±0.251	12.093±0.236	10.839±0.212
3-4	24.645±0.942	83.604±1.568	11.775±0.267	55.082±0.898	58.873±1.097	67.513±1.219	26.874±0.573	11.904±0.278	13.177±0.262	11.752±0.235

BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

Table 7: Least square means and their standard errors (LSmean±S.E.) for morphological traits as affected by sex

Sex	Morphological measurements									
	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
Male	19.155±0.809	75.548±1.346	11.739±0.230	51.585±0.770	54.513±0.941	59.976±1.047	24.244±0.492	10.328±0.239	12.572±0.225	11.120±0.202
Female	21.309±0.655	77.965±1.091	11.147±0.186	50.822±0.624	52.404±0.763	64.549±0.848	24.946±0.399	10.783±0.194	11.800±0.182	10.968±0.164

BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

4.2 DNA Polymorphisms and Haplotype Patterns of the *Melanocortin 1 Receptor* (*MC1R)* Gene

4.2.1 DNA polymorphisms and diversity in the *MC1R* gene in Nigerian and American goats

The PCR primers designed according to the sequence submitted to the EMBL database with FM212940 accession number produced a 687 bp fragment (Figure 15) spanning part of the coding region of the *MC1R* gene. The predicted protein sequence of the coding portion of this fragment contains 229 amino acids. A total of 3 polymorphisms in West African Dwarf goats; 6 in Red Sokoto goats; and 8 in Sahel goats were identified from the *MC1R* sequences, giving a frequency of one variant per 229 bp, 115 bp, and 86 bp, respectively (Table 8). The estimated nucleotide diversity within each breed varied from 1.19×10^{-3} in Red Sokoto to 1.64×10^{-3} in Sahel. Nucleotide diversity in West African Dwarf was 1.48×10^{-3}. The number of haplotypes varied for each breed. For the West African Dwarf, the number of haplotypes was 4. In both Red Sokoto and Sahel, the number of haplotypes was 7 (Table 8). Haplotype diversity (*Hd*) was highest in Sahel (0.570), followed by Red Sokoto (0.509) and then West African Dwarf (0.421). Haplotype diversities were found to be roughly similar in both Sahel and Red Sokoto. Tajima's *D*, a neutrality test, was employed to assess whether signatures of selection were present in the locus within the different breeds. The result of the test is also shown in Table 8. There were non-significant deviations from the neutral model, which assumes neutrality and random mating, for all the breeds (West African Dwarf breed = -0.89756; Red Sokoto = -1.33610 and Sahel = -1.39525).

For the American breeds, a total of 8 polymorphisms, giving a frequency of one variant per 86 bp, were identified in the Alpine breed; 3, giving a frequency of one variant per 229 bp, in the Spanish breed; and 1, giving a frequency of one variant per 687 bp, in the Saanen breed (Table 9). The estimated nucleotide diversities were 2.71 for the Alpine breed, 1.94 for the Spanish breed and 0.47 for the Saanen breed. The number of haplotypes was 6 for the Alpine breed, 4 for the Spanish breed and 2 for the Saanen breed (Table 9). Haplotype diversities were 0.679 for the Alpine breed, 0.731 for the Spanish breed and 0.286 for the Saanen breed. Non-significant deviation (-0.743) from Tajima's neutrality model was observed for all three breeds studied.

Table 8: DNA polymorphisms and diversity in the *MC1R* gene in Nigerian goats

Breed	No. of animals	No. of polymorphism	Nucleotide diversity (π x 10^{-3})	No. of haplotypes	Haplotype diversity	Tajima's D
Total	105	3	0.80	4	0.240	-1.016 (ns)
WAD	32	3	1.48	4	0.421	-0.898 (ns)
Red Sokoto	39	6	1.19	7	0.509	-1.336 (ns)
Sahel	34	8	1.64	7	0.570	-1.395 (ns)

ns: not significant,P > 0.10

Table 9: DNA polymorphisms and diversity in the *MC1R* gene in American goats

Breed	No. of animals	No. of polymorphism	Nucleotide diversity (π x 10^{-3})	No. of haplotypes	Haplotype diversity	Tajima's D
Total	40	5	1.54	6	0.626	-0.743 (ns)
Alpine	20	8	2.71	6	0.679	-0.892 (ns)
Spanish	13	3	1.94	4	0.731	0.276 (ns)
Saanen	7	1	0.47	2	0.286	-1.006 (ns)

ns: not significant, P > 0.10

4.2.2. Single Nucleotide Polymorphism (SNP) Identification

Three single nucleotide polymorphisms (SNPs) were identified upon aligning, analyzing and comparing the obtained sequence electropherograms of Nigerian goats (Figure 16). Of the three single nucleotide polymorphisms, one is a silent mutation (*g.221T>C*) at codon 61 while two are missense mutations: 1) *g.201G>A*, causing a glutamic acid to lysine change at amino acid position 55 (p.E55K); and 2) *g.206C>A*, causing an asparagine to lysine substitution at amino acid position 56 (p.N56K).

Five single nucleotide polymorphisms were identified upon analyzing sequence electropherograms obtained from American goats. Four of these mutations are missense mutations while the fifth is a silent mutation (*g.221C>T*) at codon 61. The missense mutations include: 1) *g.194T>A*, causing an amino acid change from serine to arginine at amino acid position 52 (p.S52R); 2) *g.201G>A*, causing a glutamic acid to lysine substitution at position 55 (p.E55K); 3) *g.714A>G*, causing a lysine to glutamic acid change at position 226 (p.K226E); and 4) *g.724G>C*, causing an arginine to proline change at position 229 (p.R229P).

The three mutations identified in Nigerian goats are found in the first transmembrane domain (Figure 17). Of the three mutations unique to the American goats, one is found in the first transmembrane domain (p.S52R), one in the third intracellular loop (p.K226E) and one in the sixth transmembrane domain (p.R229P).

Alignments of the deduced goat protein regions around the polymorphic sites with the corresponding MC1R amino acid positions available in other species are reported in Figures 18 and 19. The two missense mutations identified in Nigerian goats are in highly conserved positions across species. There is, however, a substitution from alanine to threonine in horse and alanine to valine in rabbit and zebra fish at the position of the silent mutation identified in this study. Of the three missense mutations unique to the American goats, two (p.S52R and p.K226E) are in highly conserved positions. An arginine to histidine substitution is however seen at position 229 in pig, horse, dog and fox. Estimation of the likelihood of the non-synonymous (missense) coding SNPs to cause a putative functional impact on the protein was attempted using the cSNP analysis tool of PANTHER (Protein Analysis THrough Evolutionary Relationships) classification system, which calculates the subPSEC (substitution Position-Specific Evolutionary Conservation) and probability ($P_{deleterious}$) scores based on an alignment of evolutionarily related proteins (Thomas *et al.,* 2003; Thomas *et al.,* 2006). The subPSEC and $P_{deleterious}$ scores were, however, not generated for all the missense mutations except the p.R229P. This is because the substitutions occur at positions that do not appear in the multiple sequence alignment. The substitutions occur at positions that are inserted relative to the consensus HMM (Hidden Markov Model) for the given family. The subPSEC and $P_{deleterious}$ scores for p.R229P are -0.99438 and 0.11861, respectively.

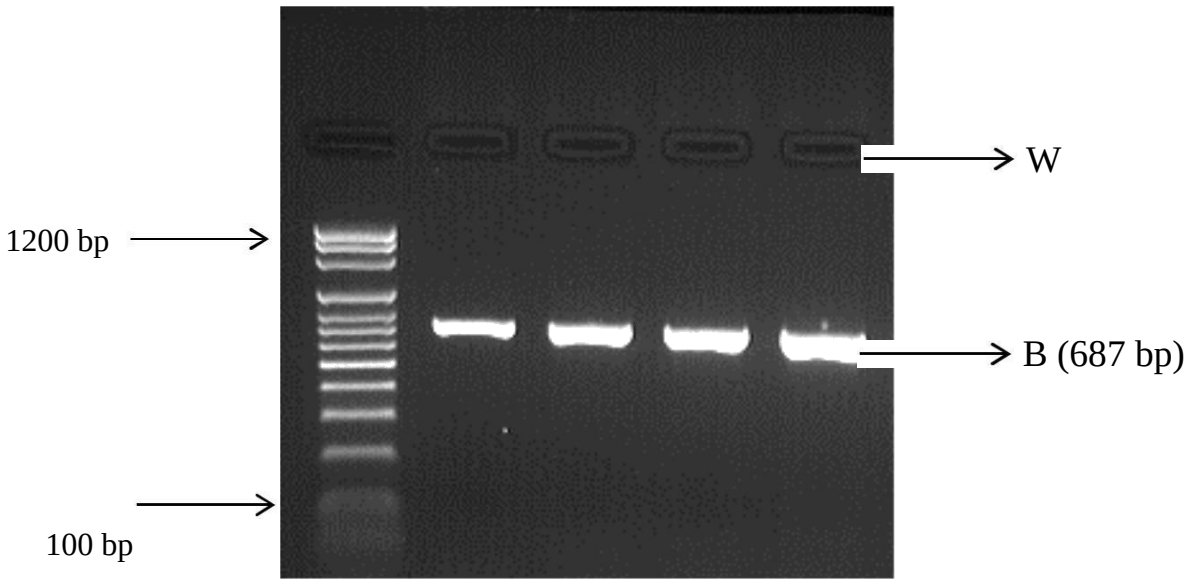

Figure 15: Amplified fragments of the *MC1R* gene from randomly selected DNA samples. The first lane from the left contains the DNA marker. W = Well (points that PCR products were loaded) casted on gel. B= Bands of amplified DNA fragments.

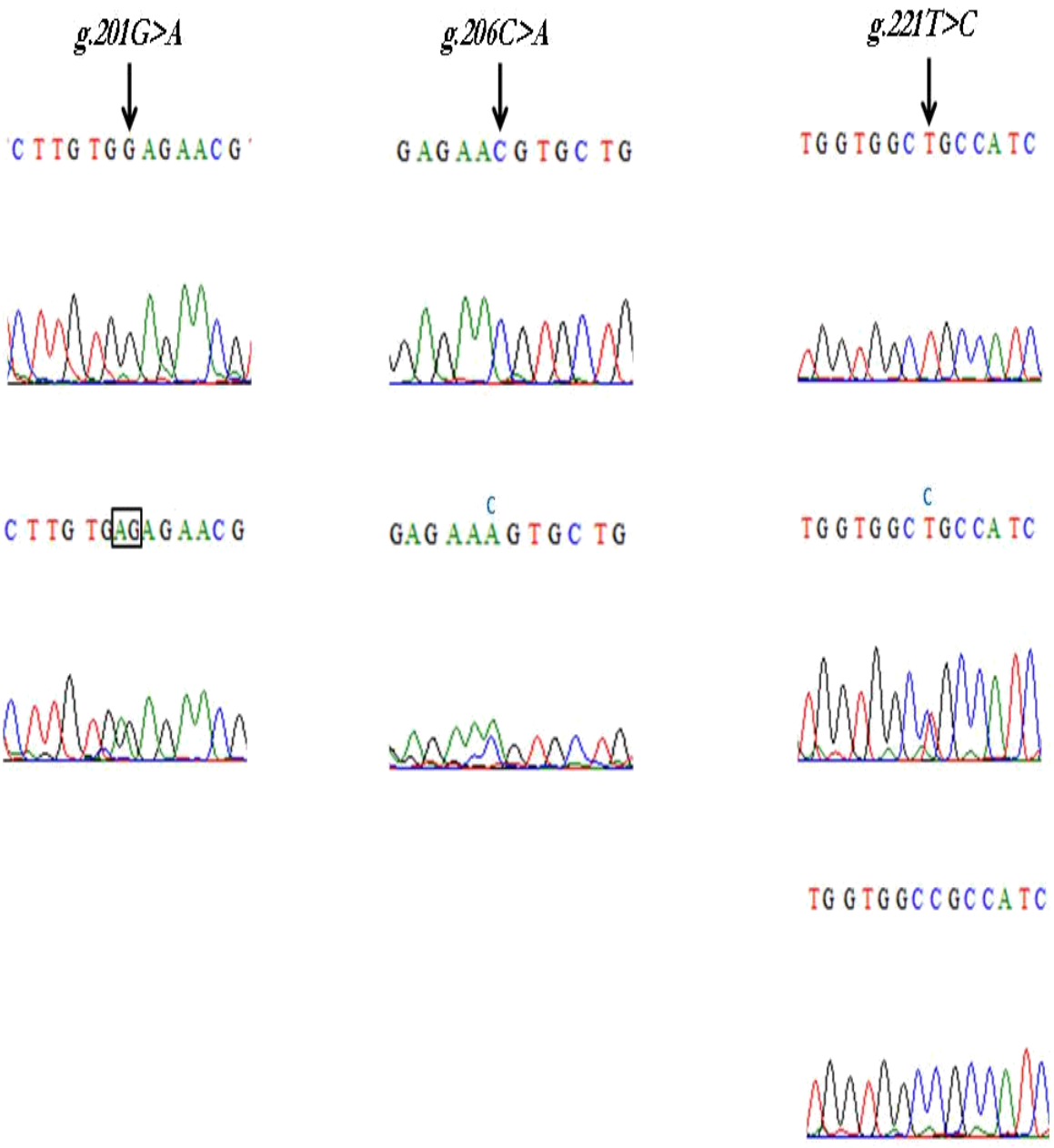

Figure 16: Sequence electropherograms showing the three SNPs reported in Nigerian goats both for the two homozygous and the heterozygous genotypes. Arrows indicate point of nucleotide substitutions. The topmost row represents homozygous genotypes for the wild type alleles, the middle represents heterozygous genotypes and the last row represents homozygous genotypes for the mutated alleles.

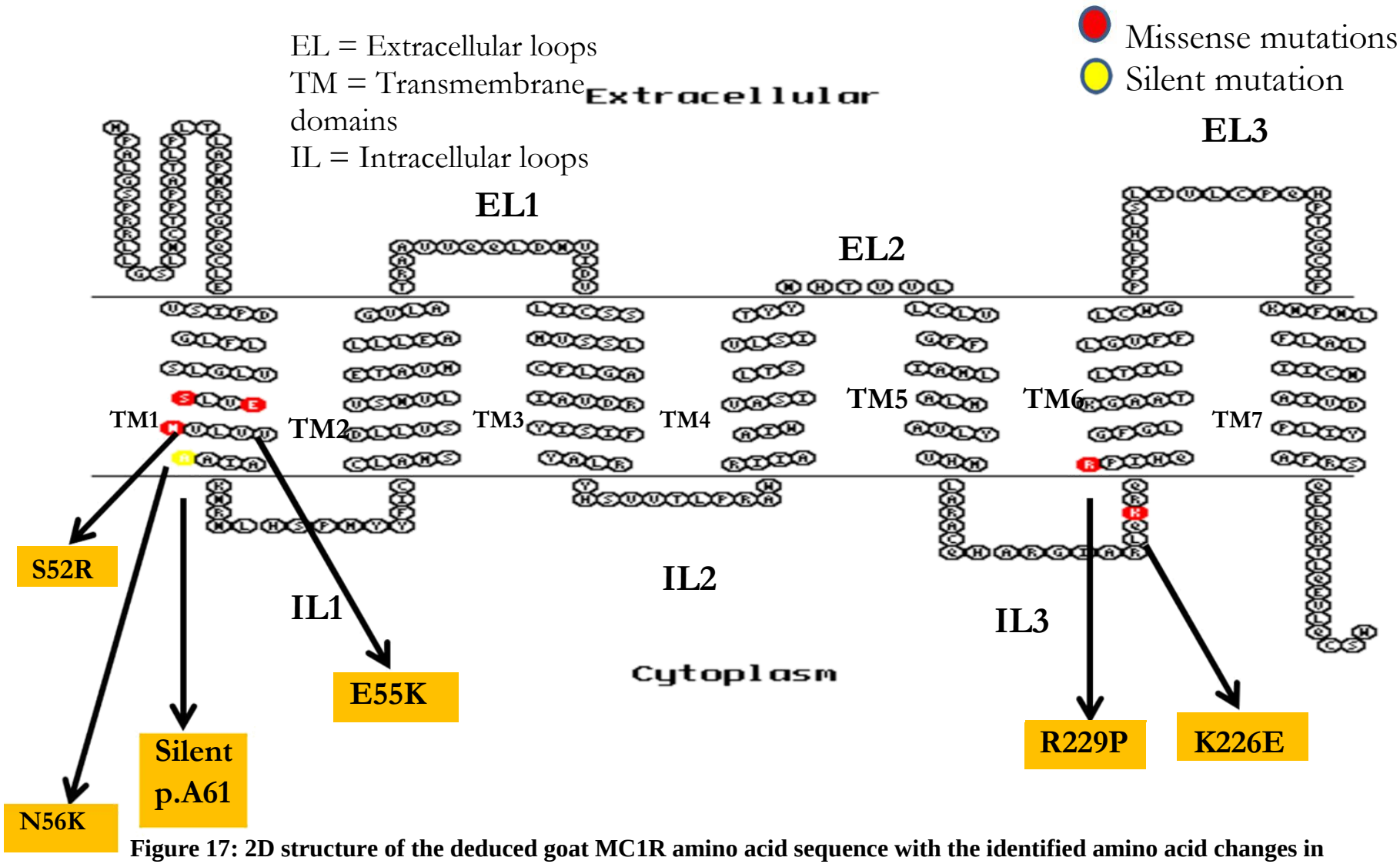

Figure 17: 2D structure of the deduced goat MC1R amino acid sequence with the identified amino acid changes in both Nigerian and American goats indicated.

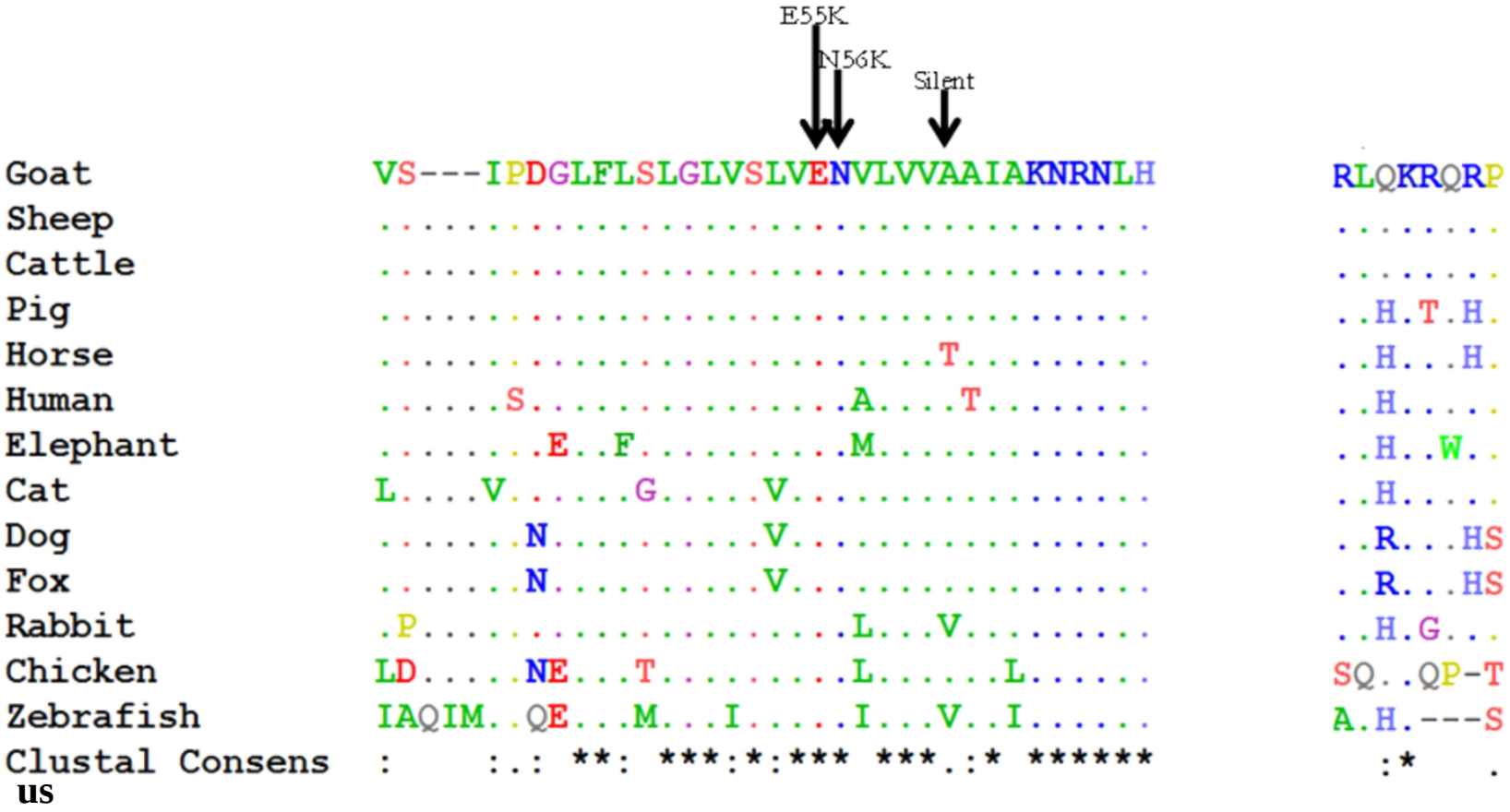

Figure 18: Alignment of the goat MC1R amino sequence regions around the corresponding position of the mutated nucleotides in Nigerian goats with the same protein regions of other species (GenBank accession numbers: sheep, [CAA74298]; cattle, [CAB64818]; pig, [NP_001008690]; horse, [NP_001108006]; human, [NP_002377]; elephant, [ABG37018]; cat, [NP_001009324]; dog, [AAC33737]; fox, [CAA62349]; rabbit, [CAJ57383]; chicken, [BAD91484]; zebrafish, [AAO24742]). **Dots indicate the same amino acid as that of the goat protein**. See Appendix 12 for amino acids and their abbreviations.

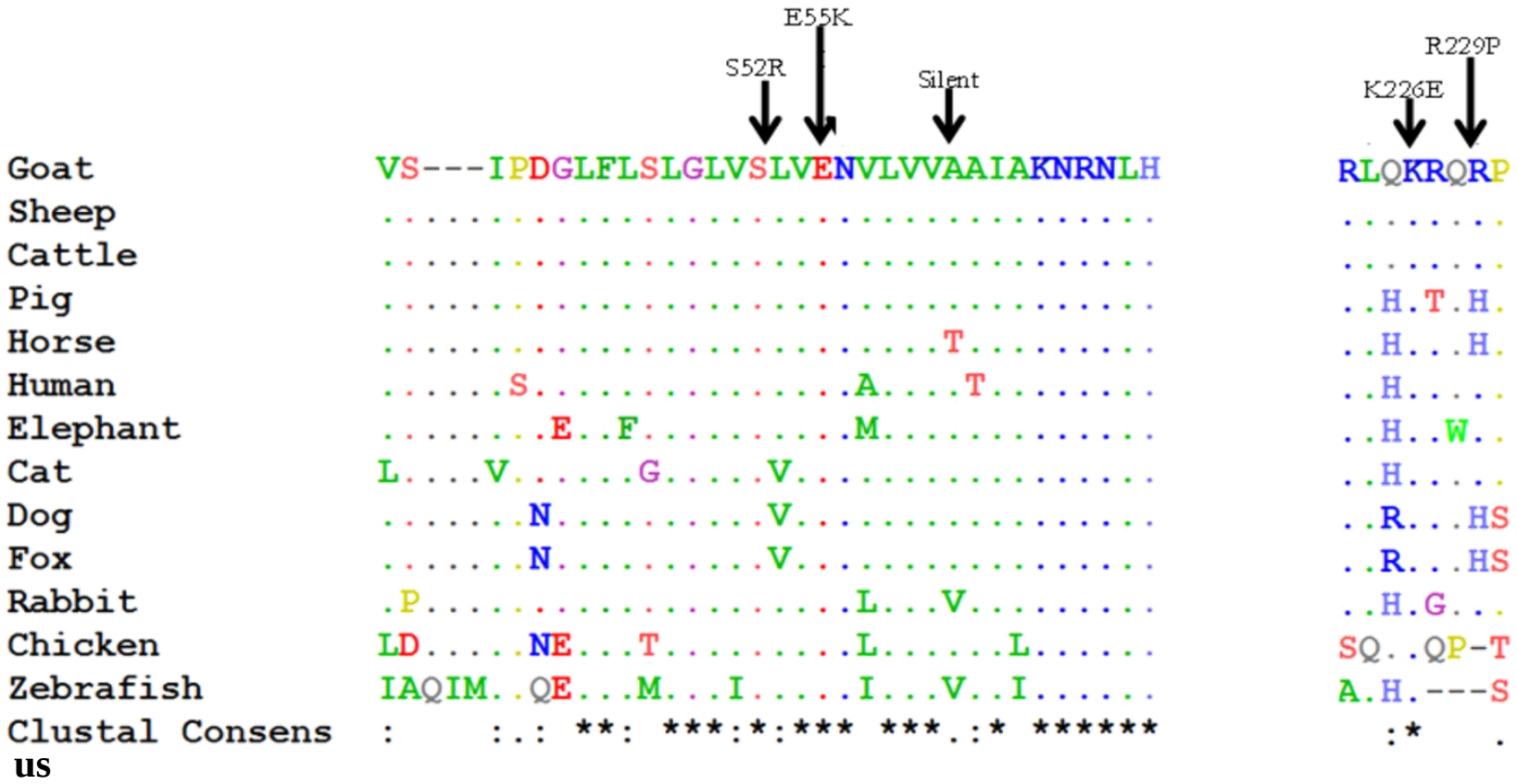

Figure 19: Alignment of the goat MC1R amino sequence regions around the corresponding position of the mutated nucleotides in American goats with the same protein regions of other species (GenBank accession numbers: sheep, [CAA74298]; cattle, [CAB64818]; pig, [NP_001008690]; horse, [NP_001108006]; human, [NP_002377]; elephant, [ABG37018]; cat, [NP_001009324]; dog, [AAC33737]; fox, [CAA62349]; rabbit, [CAJ57383]; chicken, [BAD91484]; zebrafish, [AAO24742]). Dots indicate the same amino acid as that of the goat protein. See Appendix 12 for amino acids and their abbreviations.

4.2.3 Analysis of the identified goat *MC1R* mutations in different goat breeds

Tables 10 and 11 report the genotypic frequencies for the polymorphic sites in Nigerian and American goats, respectively. Tables 12 and 13 show haplotype frequencies among the investigated Nigerian and American goat breeds, respectively. Four and six haplotypes were identified among the Nigerian and American goats, respectively. The median-joining networks showing their relationships are reported in Figure 20.

Among the Nigerian goat breeds, the missense mutations p.E55K and p.N56K present in haplotypes 3 and 4 respectively are specific to the West African Dwarf breed. The West African Dwarf goats belonging to these haplotypes are black in colour. All six goats belonging to these haplotypes, four to haplotype 3 and two to haplotype 4, are heterozygous for these mutations.

All the Red Sokoto goats used in this study belong to haplotype 1. About 82.4% of the Sahel goats belong to haplotype 1 while 17.6% belong to haplotype 2, which carries the silent mutation (*g.221*T>C). About 15.6% of the West African Dwarf goats also belong to haplotype 2. The silent mutation was found in both homozygous and heterozygous conditions in the West African Dwarf and Sahel goats.

Among the American goat breeds, haplotype 3 which carries the p.S52R and haplotype 5 which carries both the p.S52R and p.E55K seem to be specific to the Alpine breed. These mutations are present only in the heterozygous conditions. About 50% of the Alpine goats are heterozygous for the p.S52R mutation.

Haplotype 4 of the American goats carries the p.K226E while haplotype 6 carries the p.K226E and p.R229P mutations. Both mutations were found to be specific to the Spanish breed, brown and white phenotype. Only the p.K226E had the homozygous condition. This mutation was found with relatively high frequency (25%). The p.R229P was found with very low frequency (2.5%). The silent mutation, *g.221T>C*, was also found to occur with relatively high frequency in American bcreeds (32.5%). Haplotype 1 also seems to be the wild type haplotype in these goat breeds.

Table 10: Genotypic frequencies of the identified *MC1R* SNPs in the three Nigerian goat breeds

SNP	Breed (no. of animals)	Genotypic frequency (no. of animals)		
p.E55K	WAD (32)	GG=0.88(28)	GA=0.12(4)	AA=0.00(0)
(*g.201G>A*)	RS (39)	GG=1.00(39)	GA=0.00(0)	AA=0.00(0)
	Sahel (34)	GG=1.00(34)	GA=0.00(0)	AA=0.00(0)
p.N56K	WAD (32)	CC=0.94(30)	CA=0.06(2)	AA=0.00(0)
(*g.206C>A*)	RS (39)	CC=1.00(39)	CA=0.00(0)	AA=0.00(0)
	Sahel (34)	CC=1.00(34)	CA=0.00(0)	AA=0.00(0)
p.A61	WAD (32)	TT=0.50(16)	TC=0.41(13)	CC=0.09(3)
(*g.221T>C*)	RS(39)	TT=1.00(39)	TC=0.00(0)	CC=0.00(0)
	Sahel (34)	TT=0.56(19)	TC=0.41(14)	CC=0.03(1)

WAD = West African Dwarf, RS = Red Sokoto

Table 11: Genotypic frequencies of the identified *MC1R* SNPs in the three American goat breeds

SNP	Breed (no. of animals)	Genotypic frequency (no. of animals)		
p.S52R	Alpine (20)	TT=0.50(10)	TA=0.50(10)	AA=0.00(0)
(*g.194T>A*)	Spanish (13)	TT=1.00(13)	TA=0.00(0)	AA=0.00(0)
	Saanen (7)	TT=1.00(7)	TA=0.00(0)	AA=0.00(0)
p.E55K	Alpine (20)	GG=0.90(18)	GA=0.10(2)	AA=0.00(0)
(*g.201G>A*)	Spanish (13)	GG=1.00(13)	GA=0.00(0)	AA=0.00(0)
	Saanen (7)	GG=1.00(7)	GA=0.00(0)	AA=0.00(0)
p.A61	Alpine (20)	TT=0.50(16)	TC=0.15(3)	CC=0.05(1)
(*g.221T>C*)	Spanish (13)	TT=1.00(5)	TC=0.38(5)	CC=0.23(3)
	Saanen (7)	TT=0.56(6)	TC=0.14(1)	CC=0.00(0)
p.K226E	Alpine (20)	AA=1.00(20)	AG=0.00(0)	GG=0.00(0)
(*g.714A>G*)	Spanish (13)	AA=0.39(5)	AG=0.46(6)	GG=0.15(2)
	Saanen (7)	AA=0.71(5)	AG=0.29(2)	GG=0.00(0)
p.R229P	Alpine (20)	GG=1.00(20)	GC=0.00(0)	CC=0.00(0)
(*g.724G>C*)	Spanish (13)	GG=0.92(12)	GC=0.08(1)	CC=0.00(0)
	Saanen (7)	GG=1.00(7)	GC=0.00(0)	CC=0.00(0)

Table 12: Haplotype frequencies at the *MC1*R locus in Nigerian goats

Breed (no. of animals)	Coat colour	Haplotype frequency[1]			
		1 GCT	2 GCC	3 ACT	4 GAT
WAD (32)	Variety of colours	0.750	0.156	0.063	0.031
Red Sokoto (39)	Usually red	1.000	0.000	0.000	0.000
Sahel (34)	Usually white	0.824	0.176	0.000	0.000

[1]Haplotypes are indicated following the SNP position in the *MC1R* gene: *g.201G>A, g.206C>A, g.221T>C.*

Table 13: Haplotype frequencies at the *MC1*R locus in American goats

Breed (no. of animals)	Coat colour	Haplotype frequency[1]					
		1TGCAG	2TGTAG	3AGCAG	4TGCGG	5AACAG	6TGCGC
Alpine(20)	Black and white	0.550	0.150	0.250	0.000	0.050	0.000
Spanish(13)	Brown and white	0.385	0.385	0.000	0.153	0.000	0.077
Saanen (7)	White	1.000	0.000	0.000	0.000	0.000	0.000

[1]Haplotypes are indicated following the SNP position in the *MC1R* gene: *g.194T>A, g.201G>A, g.221C>T, g.714A>G, g.724G>C.*

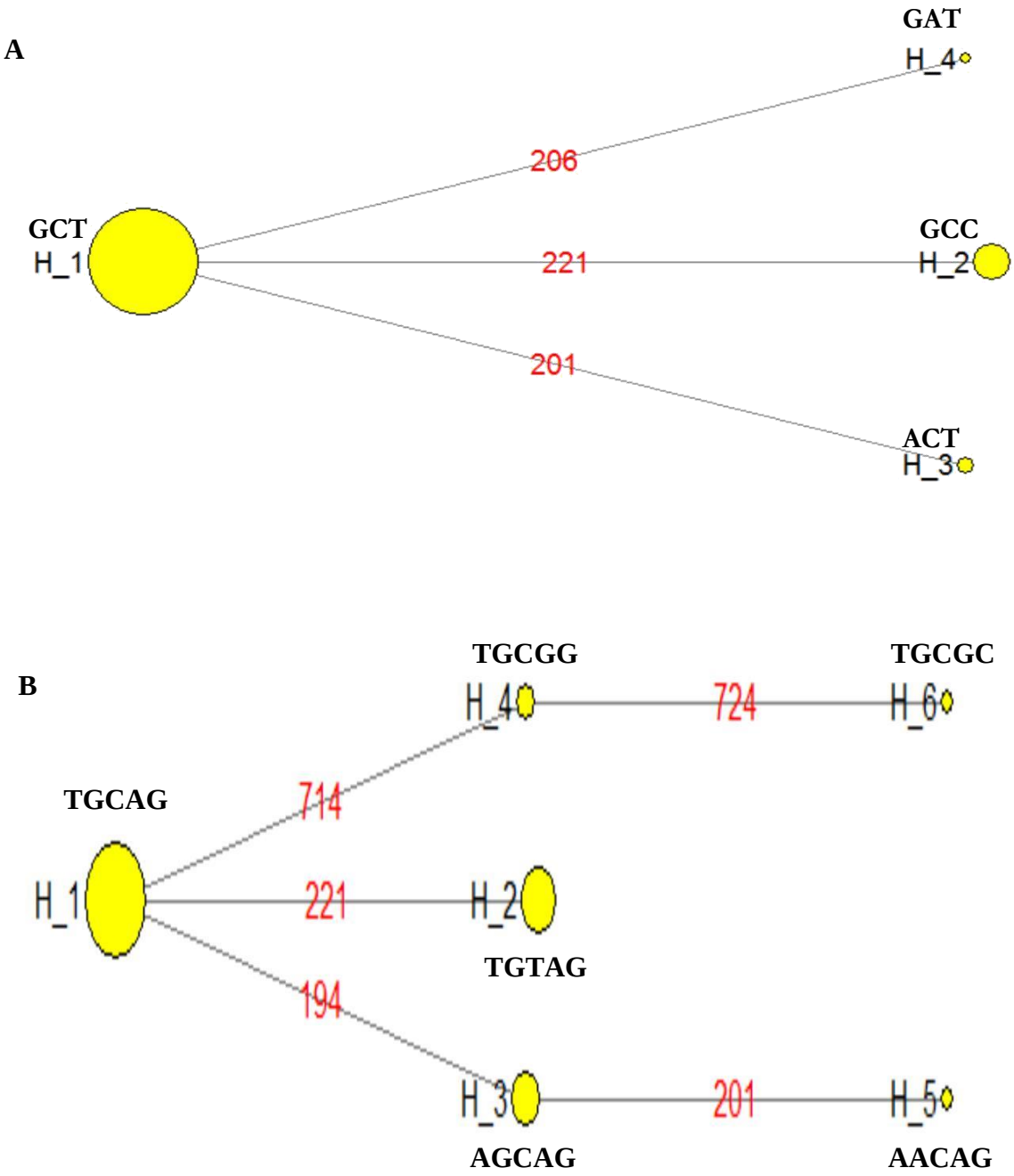

Figure 20: Median-joining network showing relationships among A) Nigerian goats *MC1R* haplotypes B) American goats *MC1R* haplotypes. The yellow circles indicate the goat haplotypes. Branch length is proportional to the number of mutations and size of node is proportional to the frequency of the haplotype. The numbers on the branches are the nucleotide positions of the mutations.

4.3 DNA Polymorphisms and Haplotype Patterns of the *Agouti Signaling Protein* (*ASIP)* Gene

4.3.1 DNA polymorphisms and diversity in the *ASIP* gene in Nigerian and American goats

The PCR primers designed according to the report of Fontanesi *et al.* (2009a) produced a 352 bp fragment (Figure 21) spanning part of exon 2 and part of intron 2. The predicted protein sequence of the coding region of this fragment contains 53 amino acids. A total of 21 polymorphisms in West African Dwarf goats; 50 in Red Sokoto goats; and 4 in Sahel goats were identified from the *ASIP* sequences, giving a frequency of one variant per 17 bp, 7 bp, and 88 bp, respectively (Table 14). Overall, 58 polymorphisms were observed and of these, only 9 were parsimony informative singleton variables. The estimated nucleotide diversity within each breed varied from 1.37×10^{-3} in Sahel to 7.83×10^{-3} in Red Sokoto. Nucleotide diversity in West African Dwarf was 4.90×10^{-3}. The number of haplotypes varied in each breed. For the West African Dwarf, the number of haplotypes was 7, 13 for Red Sokoto and 2 for Sahel (Table 14). Haplotype diversity (*Hd*) was highest in Red Sokoto (0.245), followed by West African Dwarf (0.214) and then Sahel (0.063). The result of Tajima's D neutrality test is also shown in Table 14. There were significant deviations from the neutral model for all the breeds (West African Dwarf breed = -2.529; Red Sokoto = -2.789 and Sahel = -1.889).

For the American breeds, a total of 2 polymorphisms, giving a frequency of one variant per 176 bp, were identified in the Alpine breed, none in the Spanish and Saanen breeds (Table 15). The estimated nucleotide diversity was 1.4 for the Alpine breed and the

number of haplotypes was 3 for the Alpine breed and 1 for both the Spanish and Saanen breeds (Table 9). Haplotype diversity (*Hd*) was 0.362 in the Alpine breed and non-significant deviation (-1.00161) from Tajima's neutrality model was observed for the Alpine breed.

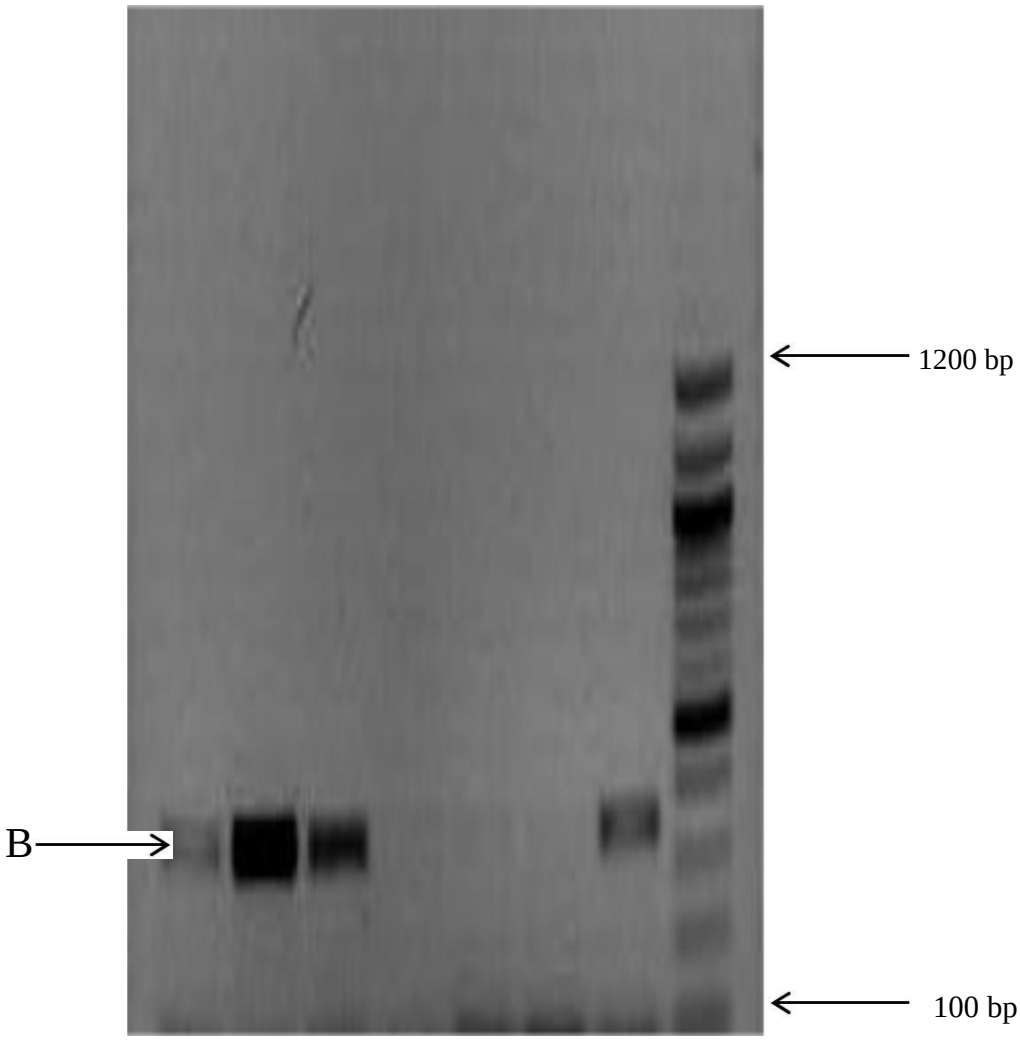

Figure 21: Amplified fragments of the *ASIP* gene from selected DNA samples. The first lane from the right contains the DNA marker. B= Bands of amplified DNA fragments

Table 14: DNA polymorphisms and diversity in the *ASIP* gene in Nigerian goats

Breed	No. of animals	No. of polymorphism	Nucleotide diversity (π x 10^{-3})	No. of haplotypes	Haplotype diversity	Tajima's D
Total	186	58	5.740	20	0.204	-2.783***
WAD	62	21	4.90	7	0.214	-2.529***
Red Sokoto	92	50	7.83	13	0.245	-2.789***
Sahel	32	4	1.37	2	0.063	-1.889*

*** significant at P < 0.001, * significant at P < 0.05

Table 15: DNA polymorphisms and diversity in the *ASIP* gene in American goats

Breed	No. of animals	No. of polymorphism	Nucleotide diversity (π x 10^{-3})	No. of haplotypes	Haplotype diversity	Tajima's D
Total	29	2	0.75	3	0.197	-1.249(ns)
Alpine	15	2	1.40	3	0.362	-1.002 (ns)
Spanish	7	0	0.00	1	0.000	nd
Saanen	7	0	0.00	1	0.000	nd

ns: not significant, P > 0.10; nd: not determined

4.3.2 Single Nucleotide Polymorphism (SNP) Identification

Nine single nucleotide polymorphisms (SNPs) were identified upon aligning the obtained sequences of Nigerian goats. Three of these single nucleotide polymorphisms are intronic mutations (*g.261C>G*; *g.280C>G*; *g.293G>A*) while the remaining six are exonic mutations. Of the six exonic mutations, five are missense mutations while one is a silent mutation (*g.173G>A;* p.K29). The exonic mutations include: 1) *g.143T>A,* causing a serine to arginine change at position 19 (p.S19R); 2) *g.191C>G*, causing an asparagine to lysine substitution at position 35 (p.N35K); 3) *g.192C>G,* causing a change from leucine to valine at position 36 (p.L36V); 4) *g.210A>C*, causing a methionine to leucine change

at position 42 (p.M42L); and 5) *g.220T>G*, causing a change from leucine to tryptophan at position 45 (p.L45W).

Two single nucleotide polymorphisms were identified upon aligning sequences obtained from American goats. Both mutations are intronic mutations and they include: *g.293G>A* and *g.327C>A.* The *g.293G>A* mutation is common to both the American and Nigerian populations.

Four of the five missense mutations identified among the Nigerian goats are present in the ASIP basic amino-terminal domain while the p.S19R mutation is present within the signal sequence (Figure 22). Among the amino acid substitutions, only lysines at positions 36 and 45 are in highly conserved positions of the mammalian ASIP basic amino-terminal domain (Figure 22). *In silico* functional analysis using PANTHER also revealed relatively high subSPEC and $P_{deleterious}$ values for these mutations, -2.44629 and 0.365 for p.L36V and -4.88323 and 0.86798 for p.L45W, respectively. The p.L45W seems to be more deleterious, having greater than 0.5 probability of causing a putative functional impact on the protein. The other mutations at the basic amino-terminal domain include p.N35K and p.M42L. The subSPEC and $P_{deleterious}$ values as predicted by PANTHER are -1.60749 and 0.19901 for p.N35K and -1.91975 and 0.25346 for p.M42L, respectively.

From the alignment of part of the goat ASIP protein with the same protein regions of other species, serine at position 19 was found only in goat and sheep. Serine is replaced by threonine in cattle, pig, horse, human, cat, dog, fox, mouse and rabbit. Serine is, however, substituted with arginine in some Nigerian goats, as identified in this study. *In*

silico functional analysis of the p.S19R mutation using PANTHER yielded subPSEC and $P_{deleterious}$ values of -1.95244 and 0.25969, respectively. These values indicate a likely non-deleterious effect of this missense mutation.

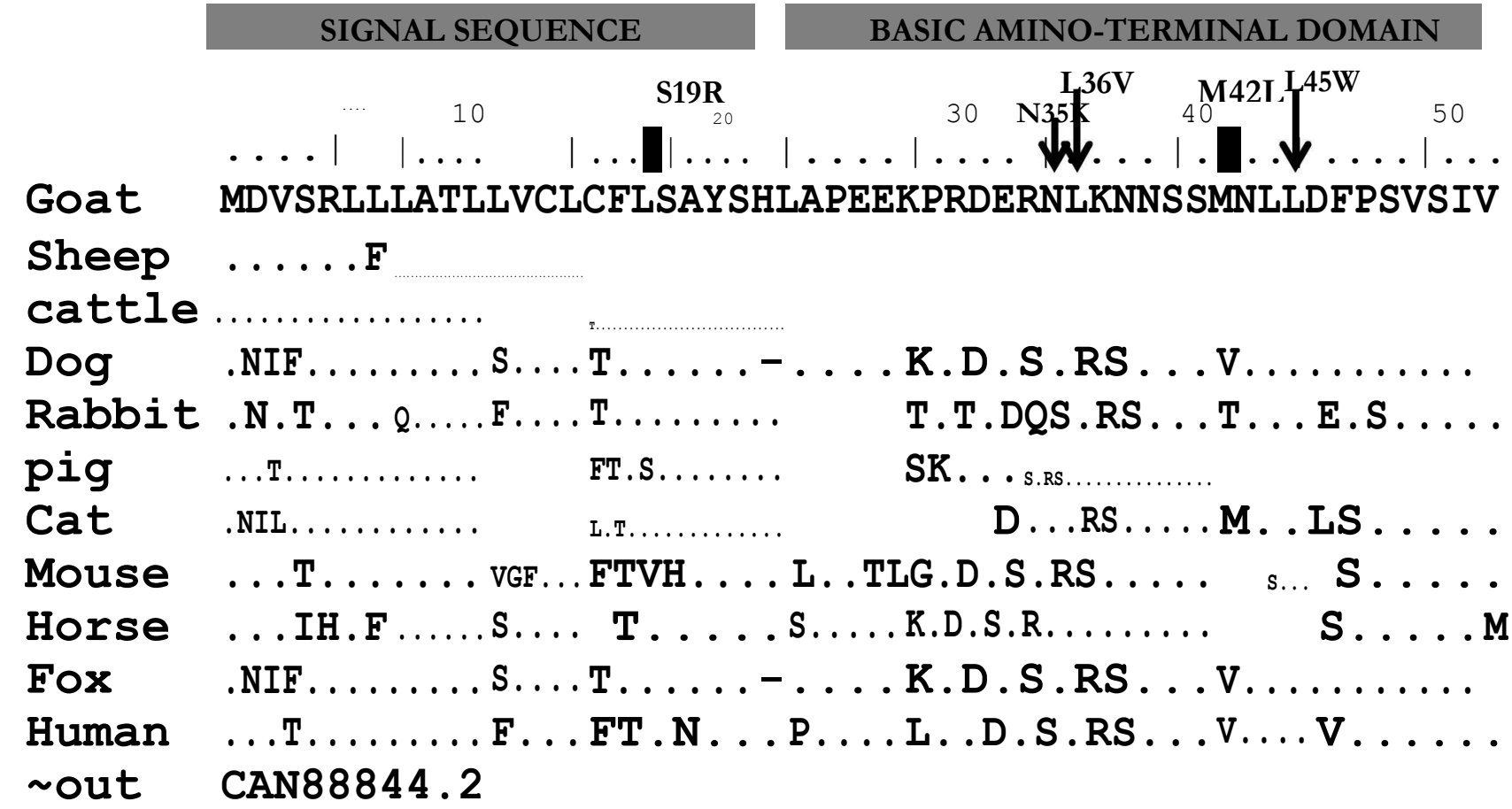

Figure 22: Alignment of part of the goat ASIP protein with the same protein regions of other species (Accession numbers: sheep, (NP_001127775.1); cattle, (AAQ56605.2); pig, (CAD20602.1); horse, (AAK70925.1); human, (AAI04240.1); cat, (AAO62411.1); dog, (ABC69425.1); fox, (P79407.2); mouse, (NP_056585.2); rabbit, (NP_001116411.1).

4.3.3 Analysis of the identified goat *ASIP* mutations in different goat breeds

Twenty and three haplotypes were identified among the Nigerian and American goats, respectively, and median-joining networks showing their relationships, are reported in Figures 23 and 24. Table 16 shows haplotype frequencies among the Nigerian goat breeds and Tables 17 and 18 show genotype and haplotype frequencies, respectively, among the investigated American goat breeds. About 89.2% of the Nigerian goats were found to belong to haplotype 1 which seems to be the wild type allele. With respect to the nine SNPs identified among Nigerian goats, there seems to be some overlap among the twenty haplotypes. Haplotypes 2, 3, 4, 9, 10 and 14 seem to overlap with haplotype 1 and haplotype 8 seem to overlap with haplotype 17. About 92.5% of these animals, therefore, belong to haplotype 1 and its overlaps. Apart from haplotype 1, the other haplotypes seem to occur with very low frequency as seen in Figure 23 and Table 16. All the Sahel goats involved in this study belong to haplotype 1. Apart from haplotype 1 and its overlaps which present about 89.1% of Red Sokoto goats, the remaining Red Sokoto goats belong to haplotypes 5, 7, 9, 11, 12, 13, 14, 16, 19 and 20 with frequency of 0.54% each. Also 90.3% of the West African Dwarf goats used in this study belong to haplotype 1 while the remaining West African Dwarf goats belong to haplotypes 6, 8, 15, 17 and 18.

Among the three American *ASIP* haplotypes, haplotype 1 seems to be the wild type haplotype with a frequency of 0.80 in Alpine breed, 1.00 in Spanish breed and 1.00 in Saanen breed (Table 18). Haplotypes 2 and 3, which carry the intronic mutations *g.293G>A* and *g.327C>A* respectively, have frequencies of 0.067 and 0.133,

respectively. These mutations were found to be specific to the Alpine breed though with very low frequency. All the Saanen and Sahel goats investigated in this study belong to haplotypes 1 of both populations which seem to be the wild type *ASIP* haplotype.

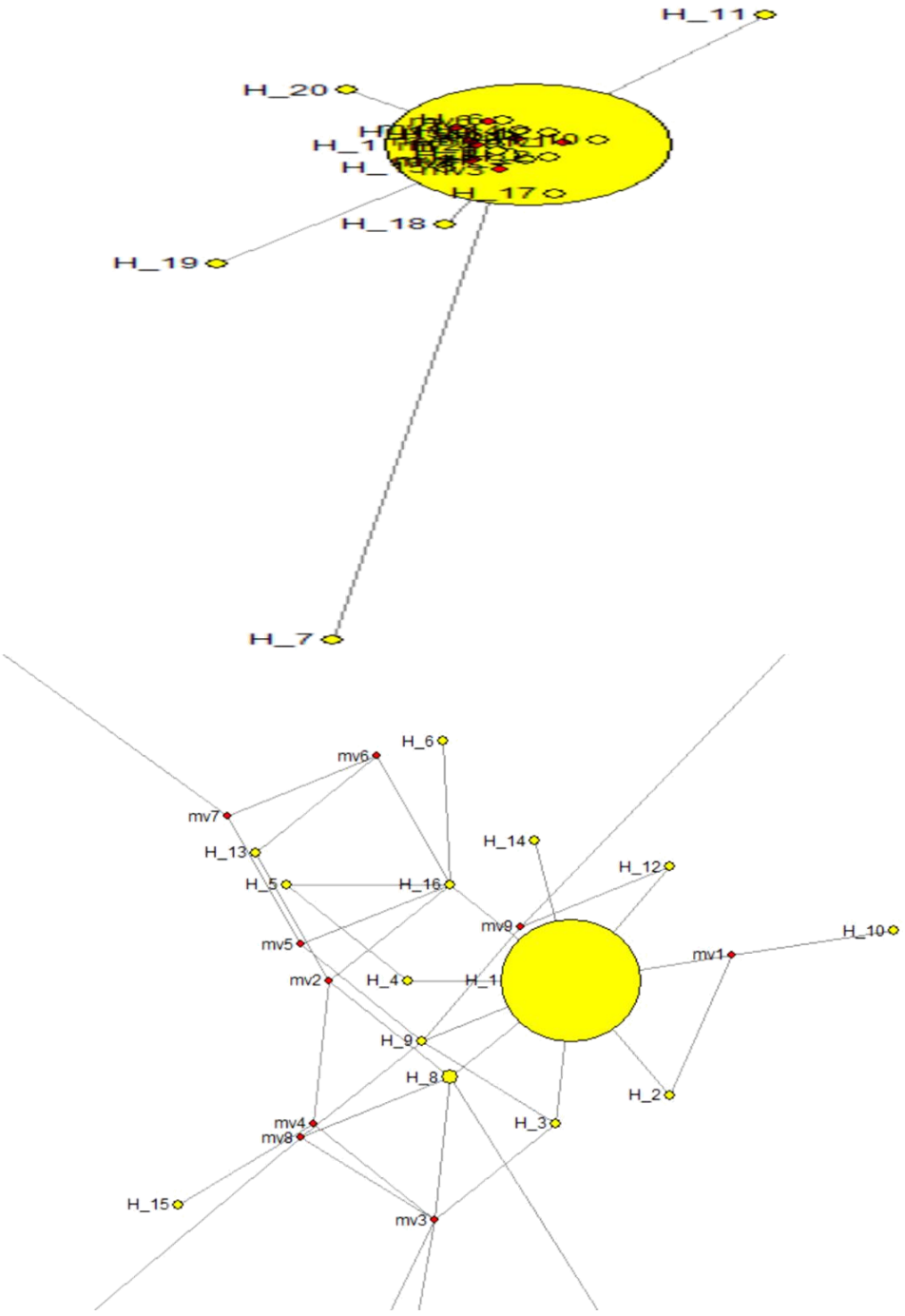

Figure 23: A) Median-joining network showing relationships among the twenty Nigerian goats *ASIP* haplotypes B) Closer view of the haplotypes excluding haplotypes 7, 11, 18, 19 and 20. The yellow circles indicate the goat haplotypes. A red square indicates the median vector (mv). Branch length is proportional to the number of mutations and size of node is proportional to the frequency of the haplotype.

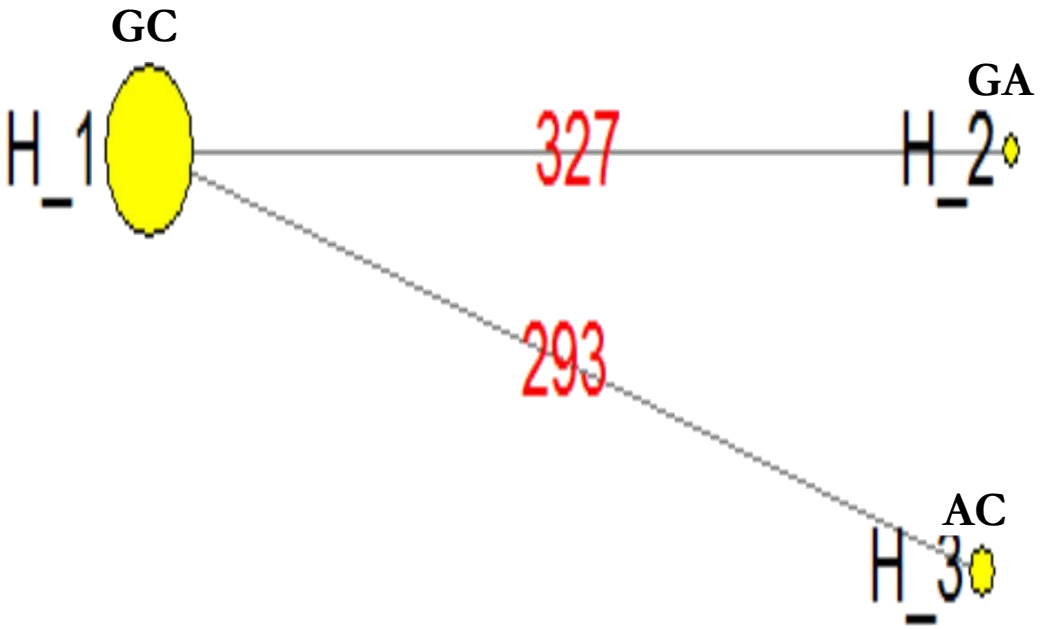

Figure 24: Median-joining network showing relationships among the three American goats *ASIP* haplotypes. The yellow circles indicate the goat haplotypes. Branch length is proportional to the number of mutations and size of node is proportional to the frequency of the haplotype. The numbers on the branches are the nucleotide positions of the mutations.

Table 16: Haplotype frequencies at the *ASIP* locus in Nigerian goats

Haplotype[1]	Frequency (number of animals)		
	West African Dwarf	Red Sokoto	Sahel
1 TGCCATCCG	0.89 (55)	0.86 (79)	1.00 (32)
2 TGCCATCCG	0.016 (1)	0.00 (0)	0.00 (0)
3 TGCCATCCG	0.00 (0)	0.011 (1)	0.00 (0)
4 TGCCATCCG	0.00 (0)	0.011 (1)	0.00 (0)
5 TGCCATCCA	0.00 (0)	0.011 (1)	0.00 (0)
6 TGCCAGCCA	0.016 (1)	0.00 (0)	0.00 (0)
7 TGCGCTGGG	0.00 (0)	0.011 (1)	0.00 (0)
8 TGCGATCCG	0.032 (2)	0.00 (0)	0.00 (0)
9 TGCCATCCG	0.00 (0)	0.011 (1)	0.00 (0)
10 TGCCATCCG	0.00 (0)	0.011 (1)	0.00 (0)
11 TACCCTGGG	0.00 (0)	0.011 (1)	0.00 (0)
12 TACCATCCG	0.00 (0)	0.011 (1)	0.00 (0)
13 TGGGATCCA	0.00 (0)	0.011 (1)	0.00 (0)
14 TGCCATCCG	0.00 (0)	0.011 (1)	0.00 (0)
15 TGCGATCCA	0.016 (1)	0.00 (0)	0.00 (0)
16 TGCCATCCA	0.00 (0)	0.011 (1)	0.00 (0)
17 TGCGATCCG	0.016 (1)	0.00 (0)	0.00 (0)
18 AGCCAGCCG	0.016 (1)	0.00 (0)	0.00 (0)
19 AGCGCGCCG	0.00 (0)	0.011 (1)	0.00 (0)
20 TGGCATCCA	0.00 (0)	0.011 (1)	0.00 (0)

[1]Haplotypes are indicated following the SNP position in the *ASIP* gene: *g.143T>A; g.173G>A; g.191C>G; g.192C>G; g.210A>C; g.220T>G; g.261C>G*; *g.280C>G*; *g.293G>A*

Table 17: Genotypic frequencies of the identified *ASIP* SNPs in the three American goat breeds

SNP	Breed (no. of animals)	Genotypic frequency (no. of animals)		
g.293G>A	Alpine (15)	GG=0.88(14)	GA=0.12(1)	AA=0.00(0)
	Spanish (7)	GG=1.00(7)	GA=0.00(0)	AA=0.00(0)
	Saanen (7)	GG=1.00(7)	GA=0.00(0)	AA=0.00(0)
g.327C>A	Alpine (15)	CC=0.94(14)	CA=0.06(1)	AA=0.00(0)
	Spanish (7)	CC=1.00(7)	CA=0.00(0)	AA=0.00(0)
	Saanen (7)	CC=1.00(7)	CA=0.00(0)	AA=0.00(0)

Table 18: Haplotype frequencies at the *ASIP* locus in American goats

Breed (no. of animals)	Coat colour	Haplotype frequency[1]		
		GC	GA	AC
Alpine (15)	Black and white	0.80	0.067	0.133
Spanish (7)	Brown and white	1.00	0.00	0.00
Saanen (7)	White	1.00	0.00	0.00

[1]Haplotypes are indicated following the SNP position in the *ASIP* gene: *g.293G>A* and *g.327C>A.*

4.4 Variation of *g.469*C>G in the *Melanophilin* (*MLPH*) Gene Intron 2 in Nigerian Goat Breeds.

4.4.1 Goat *MLPH* gene sequences and SNP identification

The PCR primers produced a 648 bp fragment (Figure 25), spanning intron 1, exon 2 and part of intron 2 (GenBank EU316218). The transversion g.*469C>G* was identified in intron 2 from aligning sequences obtained from 20 animals. This mutation was analyzed in a larger number of animals of the three Nigerian goat breeds using PCR-RFLP method. Digestion of the PCR products with *Hinp1*I yielded two fragments (532 and 116 bp) for C allele and one fragment (648 bp) for G allele. Genotypes CC, CG and GG, therefore, demonstrated two, three and one band respectively (Figure 26).

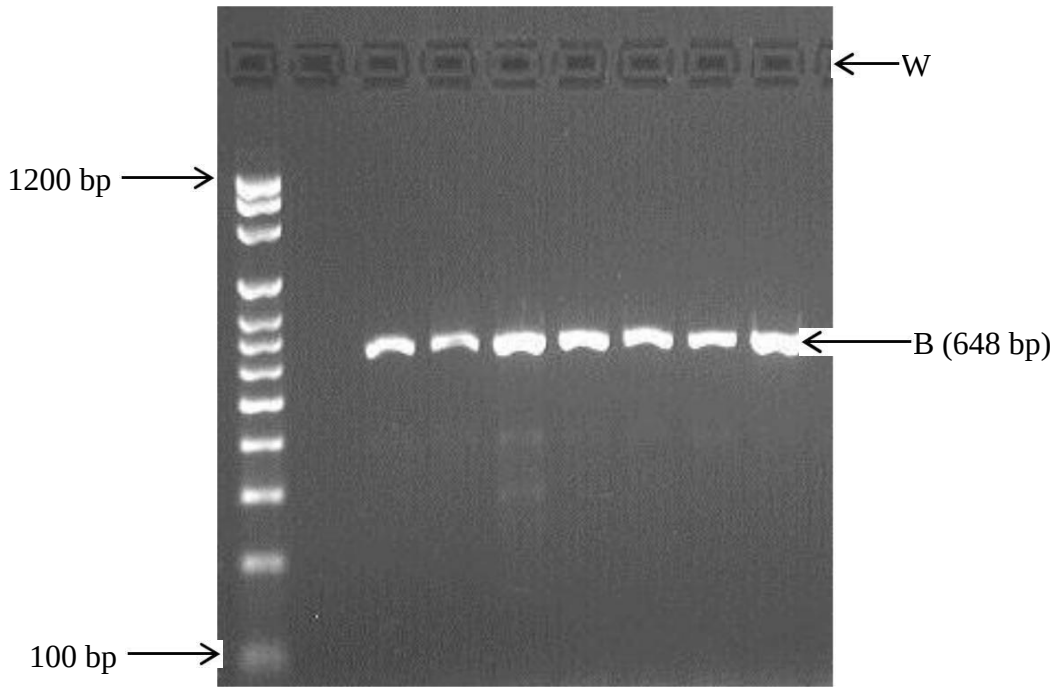

Figure 25: Amplified fragments of the *Melanophilin* gene. The first lane from the left contains the DNA marker. W = Well (points that PCR products were loaded) casted on gel. B= Bands of amplified DNA fragments.

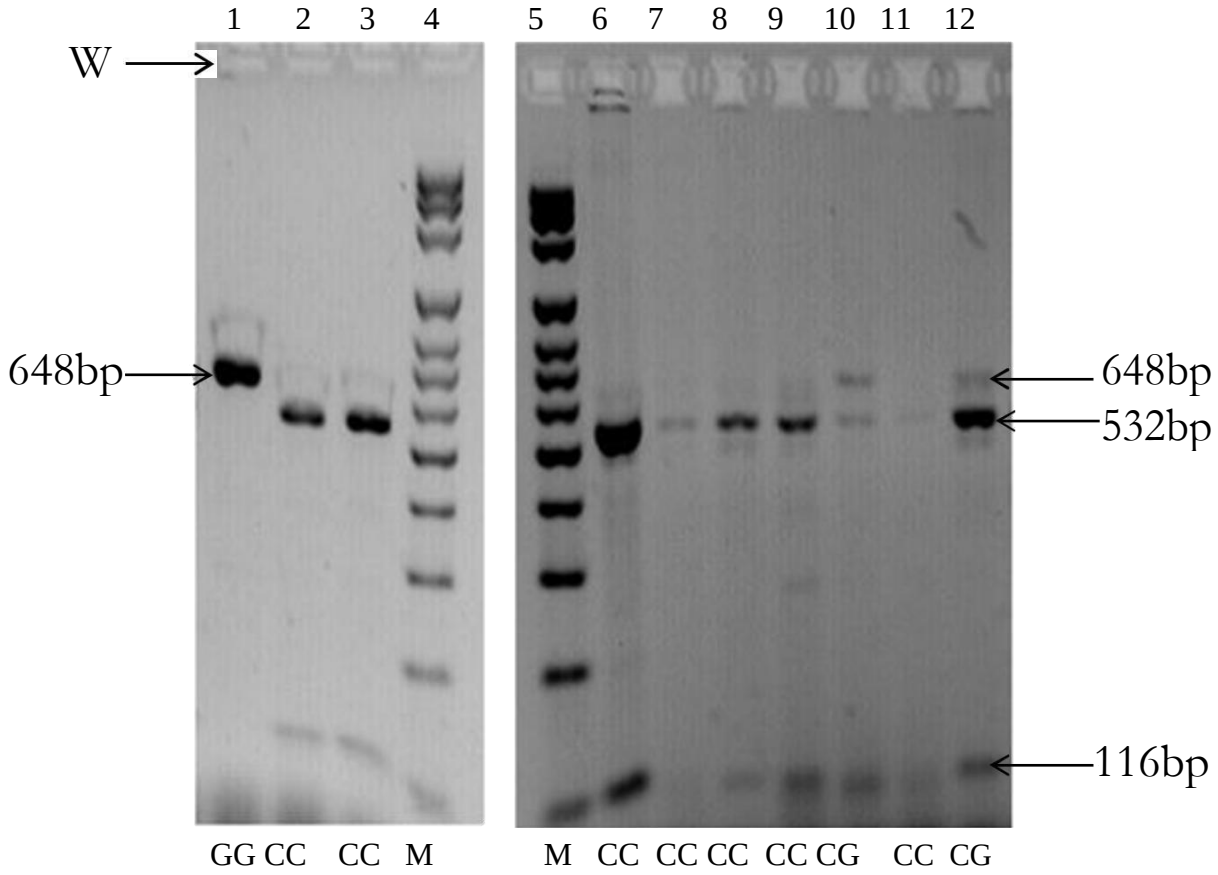

Figure 26: Result of PCR-RFLP by *Hinp1*I.

The recognition site of ***Hinp1*I** is: 5′...GCGC...3′ 3′...CGCG...5′. Digestion of the PCR products with *Hinp1*I yielded two fragments (532 and 116 bp) for C allele and one fragment (648 bp) for G allele. Genotypes CC, CG and GG, therefore, demonstrated two, three and one band respectively M refers to DNA Marker. Lane 1 illustrates genotype GG with one band of 648 bp, lanes 2, 3, 6, 7, 8, 9 and 11 illustrate genotype CC with two bands of 116 bp and 532 bp, and lanes 10 and 12 illustrate genotype CG three bands of 116 bp, 532 bp and 648 bp. Lanes 4 and 5 contain DNA marker. W = Well (points that PCR products were loaded) casted on gel.

4.4.2 The distribution of g.*469C>G* in the different Nigerian goat breeds

Genotypic frequencies, allelic frequencies and the probability of departure from Hardy-Weinberg equilibrium of the different goat breeds are shown in Table 19. Allele C had frequencies of 0.974, 0.980 and 0.963 in Red Sokoto, Sahel and West African Dwarf goats, respectively. West African Dwarf had the highest frequency (0.038) of allele G, then Red Sokoto (0.026) and then Sahel (0.020). Also, homozygous GG was found only in Red Sokoto breed. The chi-square test, based on the allelic frequencies, revealed no significant differences between the breeds for g.*469C>G* (Table 20). Both West African Dwarf and Sahel breeds appear to be in Hardy-Weinberg equilibrium at 0.05 rejection level for g.*469C>G*. The deficit of heterozygous individuals as seen in Table 18 probably accounts for the deviation from Hardy-Weinberg equilibrium for the Red Sokoto breed. Overall, it appears that the population of Nigerian goats is in Hardy-Weinberg equilibrium with respect to this locus ($P<0.05$).

Table 19: Genotype and gene frequencies of g.*469C>G* in Nigerian goat Breeds

Goat Breed	Sample size	Genotypic frequencies			Allelic frequencies		HW[c]
		CC	CG	GG	C	G	
Red Sokoto	135	0.956	0.037	0.007	0.974	0.026	0.001
Sahel	51	0.961	0.039	0.000	0.980	0.020	0.920
West African Dwarf	80	0.925	0.075	0.000	0.963	0.038	0.751
Overall	266	0.947	0.049	0.004	0.972	0.028	0.064

[c] Probability of departure from Hardy-Weinberg equilibrium

Table 20: χ^2 and P values for differences among the three Nigerian goat breeds based on allelic frequencies

	Red Sokoto	Sahel	West African Dwarf
Red Sokoto	-	0.125 (P = 0.724)	0.459 (P = 0.498)
Sahel		-	0.674 (P = 0.412)
West African Dwarf			-

4.4.3 Genetic diversity and differentiation of g.*469C>G* in different Nigerian goat breeds

Tables 21, 22 and 23 show the genetic diversity and differentiation of g.*469C>G* in Nigerian goat breeds. The observed heterozygosity ranged from 0.0370 (Red Sokoto) to 0.0750 (WAD). The Levene and Nei's expected heterozygosity ranged from 0.0388 (Sahel) to 0.0726 (WAD) and 0.0384 (Sahel) to 0.0722 (WAD), respectively. The two kinds of expected heterozygosity had similar values, with little difference from observed heterozygosity. There was, however, a somewhat large difference in the observed heterozygosity from the two kinds of expected heterozygosity in Red Sokoto. Only Red Sokoto (red coat colour) showed plus value of Wright's fixation index also indicating an excess of homozygotes in this breed. The relative degree of genetic variation of g.*469C>G* for each breed could be shown as WAD>Red Sokoto>Sahel based on the Shannon index value. All three goat breeds were observed to be low in genetic diversity at g.*469C>G*. Table 22 shows overall low Fis and Fst values and high Nm value. Table 19 also shows a high genetic identity between the breeds for g.*469C>G*. Red Sokoto appears to be more similar to Sahel than West African Dwarf.

Table 21: The heterozygosis of g.*469C>G* in different Nigerian goat breeds

Breed	Sample size	Observed value		Expected** values		Nei's H***	Ne	Fis	I
		Hom	Het	Hom	Het				
RS	135	0.963	0.037	0.949	0.051	0.051	1.053	0.267	0.120
SH	51	0.961	0.039	0.961	0.039	0.038	1.040	-0.020	0.097
WAD	80	0.925	0.075	0.927	0.073	0.072	1.078	-0.039	0.160
Overall	266	0.951	0.049	0.945	0.055	0.055	1.058	0.108	0.128

*Breed: RS = Red Sokoto; SH = Sahel; WAD = West African Dwarf. ** Expected homozygosity and heterozygosity were computed using Levene (1949); *** Nei's 1973 expected heterozygosity; Ne = effective number of alleles [Kimura and Crow (1964)]; I = Shannon's information index [(Lewontin (1972)]; Fis = Wright's fixation index

Table 22: Summary of F-Statistics and Gene Flow [Nei (1987)] of g.*469C>G* between Nigerian goat breeds

Fis	Fit	Fst	Nm
0.061	0.063	0.002	122.323

Table 23: Genetic identity [Nei (1972)] between Nigerian Goat Breeds

Population	Red Sokoto	Sahel	West African Dwarf
Red Sokoto	-	1.0000	0.9999
Sahel		-	0.9998
West African Dwarf			-

4.5 Association between identified mutations at the *MC1R* locus and some body traits in Nigerian goats

4.5.1 Association between *g.201G>A* and Body Traits

A summary description of the analyses of variance for morphological traits and *g.201G>A* genotypes is shown in Table 24. There was no variation among the genotypes with respect to this transition for all the body traits studied. Breed showed significant effect on all the body traits except body weight and rump width, and sex affected all but tail length, withers height, rump height and fore cannon bone length. The interaction between genotype and breed did not significantly influence all the morphological traits except ear length. The interaction between breed and sex did not show significant effect on all the body traits.

4.5.2 Association between *g.206C>A* and Body Traits

A summary description of the analyses of variance for morphological traits and *g.206C>A* genotypes is shown in Table 25. Genotype was found not to significantly influence any of the body traits taken. Breed significantly affected all the body traits. Sex showed significant effect on body weight, heart girth, chest depth and ear length. The interaction between breed and sex did not significantly affect all the body traits.

Table 24: Analysis of variance for morphological traits and *g.201G>A* genotypes in the three Nigerian goat breeds

Source of Variation	Mean squares and level of significance									
	DF	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
Genotype	1	200.652^{d}	433.024^{d}	0.114^{d}	487.212^{d}	7.446^{d}	95.627^{d}	13.077^{d}	8.677^{d}	4.056^{d}	9.889^{d}
Breed	2	180.018^{d}	850.681^{a}	31.811^{a}	2626.871^{a}	2403.223^{a}	199.880^{c}	66.723^{c}	3.486^{d}	140.727^{a}	21.395^{b}
Sex	1	290.53^{c}	472.211^{c}	1.494^{d}	281.125^{d}	269.010^{d}	449.182^{b}	149.710^{b}	16.119^{c}	2.527^{d}	27.497^{b}
Genotype x Breed	1	42.506^{d}	221.116^{d}	3.841^{d}	378.053^{d}	62.611^{d}	2.057^{d}	4.434^{d}	1.148^{d}	5.133^{d}	16.623^{c}
Breed x Sex	2	66.281^{d}	57.283^{d}	2.778^{d}	69.794^{d}	125.792^{d}	84.536^{d}	4.019^{d}	3.194^{d}	0.961^{d}	5.616^{d}
Residual	97	58.514	110.474	3.204	147.190	148.625	62.801	14.422	3.249	3.169	3.806

DF = Degree of freedom; BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

^{a,b,c},Significant at P<0.001, P<0.01 and P<0.05, respectively; ^{d} Not significant

Table 25: Analysis of variance for morphological traits and *g.206C>A* genotypes in the three Nigerian goat breeds

Source of Variation	Mean squares and level of significance									
	DF	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
Genotype	1	2.672^{d}	123.229^{d}	0.625^{d}	33.603^{d}	16.960^{d}	2.593^{d}	6.713^{d}	3.189^{d}	2.072^{d}	3.001^{d}
Breed	2	565.702^{a}	2718.066^{a}	46.185^{a}	2790.147^{a}	2416.993^{a}	661.056^{a}	194.539^{a}	26.551^{a}	178.352^{a}	13.537^{c}
Sex	1	239.007^{c}	371.126^{d}	2.081^{d}	223.213^{d}	250.556^{d}	392.646^{c}	138.611^{b}	12.594^{d}	2.496^{d}	28.486^{b}
Breed x Sex	2	65.156^{d}	47.486^{d}	2.050^{d}	57.297^{d}	132.955^{d}	71.197^{d}	5.168^{d}	2.158^{d}	0.731^{d}	4.844^{d}
Residual	98	59.937	112.890	3.208	151.464	147.584	63.224	14.343	3.273	3.180	3.928

DF = Degree of freedom; BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

^{a,b,c},Significant at P<0.001, P<0.01 and P<0.05, respectively; ^{d} Not significant

4.5.3 Association between *g.221T>C* and Body Traits

Table 26 shows a summary description of the analyses of variance for morphological traits and *g.221T>C* genotypes. There was no variation among genotypes in morphological traits measured. Among the morphological measurements studied, body weight, rump width and ear length were affected by variation in breed. Sex did not significantly affect any of the body traits. The interaction between breed and genotype did not significantly affect any of the body traits. Except for body length, genotype x sex interaction was not significant for all the morphological traits. The interaction between breed and sex also significantly affected none of the body traits but ear length. The interaction between all three sources of variation did not significantly influence any of the body traits.

Table 26: Analysis of variance for morphological traits and *g.221T>C* genotypes in the three Nigerian goat breeds

Source of Variation	Mean squares and level of significance									
	DF	BW	BL	TL	WH	RH	HG	CD	RW	FCL	EL
Genotype	2	56.008^{d}	118.431^{d}	0.609^{d}	245.430^{d}	196.316^{d}	33.001^{d}	10.215^{d}	0.651^{d}	1.818^{d}	7.206^{d}
Breed	2	362.055^{b}	1523.915^{a}	25.464^{a}	1287.594^{a}	1110.174^{a}	499.952^{a}	119.215^{a}	9.808^{d}	95.416^{a}	4.267^{d}
Sex	1	28.214^{d}	187.022^{d}	0.975^{d}	0.573^{d}	1.663^{d}	54.116^{d}	28.600^{d}	2.065^{d}	0.627^{d}	6.408^{d}
Genotype x Breed	3	94.484^{d}	55.556^{d}	4.352^{d}	173.970^{d}	188.200^{d}	44.686^{d}	13.482^{d}	0.691^{d}	0.082^{d}	2.474^{d}
Genotype x Sex	2	135.003^{d}	459.152^{c}	0.665^{d}	280.359^{d}	271.646^{d}	141.268^{d}	18.748^{d}	8.715^{d}	2.457^{d}	7.822^{d}
Breed x Sex	2	115.044^{d}	201.510^{d}	0.954^{d}	28.883^{d}	65.864^{d}	170.593^{d}	9.122^{d}	6.451^{d}	0.313^{d}	12.032^{c}
Genotype x Breed x Sex	2	50.150^{d}	78.329^{d}	4.790^{d}	100.440^{d}	129.066^{d}	73.552^{d}	18.436^{d}	1.948^{d}	4.502^{d}	6.676^{d}
Residual	90	59.145	111.247	3.161	153.618	146.863	64.036	14.605	3.323	3.215	3.577

DF = Degree of freedom; BW = Body weight; BL = Body length; TL = Tail length; WH = Withers height; RH = Rump height; HG = Heart girth; CD = Chest depth; RW= Rump width; FCL = Fore cannon bone length; EL = Ear length

^{a,b,c},Significant at P<0.001, P<0.01 and P<0.05, respectively; ^{d} Not significant

5.0 DISCUSSION

5.1 EFFECT OF COAT COLOUR ON SOME MORPHOLOGICAL TRAITS OF NIGERIAN INDIGENOUS GOATS

Particular morphometric traits are said to indicate inherent size as they are less affected by nutrition especially after the growing phase of animals, and these include body length, body height and chest depth (Coleman and Evans, 1985; Kamalzadeh *et al*., 1998; Semakula *et al.,* 2010). Body dimensions related to the deposition of fat and muscle include width, girth measurements and body weight. Such fat and muscle-related measurements are also affected by gut-fill and pregnancy in females (Kamalzadeh *et al*., 1998).

The results of this study showed that white animals had the least body length and rump width. Mohammed and Amin (1996) also reported the narrow body and shallow chest of Sahel goats. The observation that the white-coloured animals had the longest fore cannon bone and ear might not be surprising. The white colour is the most predominant colour of the Sahel goats which are mostly found in the Northern border of Nigeria with extreme weather conditions. This part of the country records the highest temperatures and the relatively long fore cannon bone and ear of these animals may serve as adaptive mechanisms for their survival in the very harsh and extreme weather conditions. The ears and fore cannon bones are less covered with hair when compared to the other parts of the body. These parts therefore allow the dissipation of heat from the body. The white colour of these animals also serves some adaptive functions as white colour absorbs relatively less solar radiation. Mohammed and Amin (1996) also recorded longer legs of the Sahel

goats when compared to the other breeds. The result of this study is contrary to that of Ozoje and Mgbere (2002) who noted longer legs with increase in pigmentation. It is however worth noting that Ozoje and Mgbere (2002) studied West African Dwarf goats sampled from SouthWestern Nigeria.

The lower measurements were recorded for the black-coloured animals followed by the brown-coloured for most of the body measurements. The black and brown-coloured animals in this study mostly belonged to the West African Dwarf breed. Yakubu *et al.* (2010) reported lower body measurements for the West African Dwarf goats relative to the Red Sokoto breed. Attah *et al.* (2004) also reported higher measurements for some body dimensions for the Red Sokoto goats compared to the West African Dwarf breed.

Generally the body measurements for Sahel goats were found to be slightly or marginally higher than those for Red Sokoto goats. Makun *et al.* (2008) also observed higher body weight for Sahel goats compared to the Red Sokoto goats across different ages. A similar trend with respect to the higher body weight and body measurements of Red Sokoto goats relative to West African Dwarf was also recorded by Yakubu *et al.* (2010). Yakubu *et al.* (2010) recorded similar values for body weight, withers height, rump height, heart girth and chest depth for the West African Dwarf and Red Sokoto goats. Mohammed and Amin (1996) reported similar values as those recorded in this study for withers height and body weight for the Sahel breed. The observed increase in body weight and body dimensions with increase in age is expected since size and shape are expected to increase

with age. This report is consistent with those of Fajemilehin and Salako (2008) and Semakula *et al.* (2010).

This study showed that sex is not an important source of variation for most of the body dimensions studied. Olutogun *et al*. (2003) also reported a non-significant effect of sex on body weight and linear measurements except heart girth while working on White Fulani and Gudali cattle in Nigeria. Devendra and Burns (1983), Ifut *et al.* (1991), Akpa *et al*. (1998) and Tsegaye (2009), however, reported significant influence of sex on body weight and linear traits in goats. The higher body weight and heart girth of female goats observed in this study is in agreement with that of Fajemilehin and Salako (2008) who reported higher body weight of does. They also reported greater body length, heart girth, withers height and rump height of female animals than males. The longer tail of male goats observed in this study has also been reported in sheep, chicken, turtles, snake and some other animals (Kaplan, 1994; Shine *et al.*, 1999; Yakubu *et al.*, 2011). Shine *et al.* (1999) hypothesized the following for the longer tail of male snakes: 1) tail length affects the size of the male copulatory organs (hemipenes) such that males with longer tails relative to body length have longer hemipenes presumably because of the additional space available (the hemipenes are housed inside the tail base); 2) relative tail length affects male mating success. They observed a three-fold reduction in mating success among males with partial tail loss (due to predation or misadventure) and strong stabilizing selection on relative tail length among males with intact tails from their studies. The reason for the longer tail in male goats is, however, unknown. Yakubu *et al.* (2011) also observed longer fore cannon bones in male sheep relative to female sheep.

The longer fore cannon bone of male goats observed in this study might contribute to their higher rump and withers height. Mohammed and Amin (1996), Adeyinka and Mohammed (2006), Akpa *et al.* (1998), Hamayun *et al.* (2006), Tsegaye (2009) and Semakula *et al.* (2010), however, reported higher body weight and body dimensions for male goats than female goats.

5.2 DNA POLYMORPHISMS AND HAPLOTYPE PATTERNS OF THE MELANOCORTIN 1 RECEPTOR (*MC1R)* GENE

Both nucleotide and predicted amino acid sequences of the amplified *MC1R* fragment showed 99% and 100% similarity, respectively, to the caprine *MC1R* sequences in the EMBL database with accession numbers FM212940 and CAR82366.1, respectively using the Basic Local Alignment Search Tool (BLAST) from www.pubmed.org (Appendices 5 and 6). The predicted amino acid structure from this study was found to be similar to that of Majerus and Mundy (2003).

This study identified a low level of nucleotide diversity (1.01×10^{-3}) in the portion of the *MC1R* gene studied in Nigerian goats. Similar values were obtained for the American breeds, except for the Alpine breed where a relatively high number of polymorphism and nucleotide diversity were observed. The American breeds are probably generally more polymorphic (1.54×10^{-3}) but this trend could not be seen in the Spanish and Saanen breeds possibly because of the smaller population sizes relative to the Alpine breed. Nucleotide diversity was also found to be low (1.01×10^{-3}) in a study of *MC1R* gene on

some human populations (Savage *et al.*, 2008) where nucleotide diversity was published. Among the Nigerian breeds, the Sahel goat was found to be most polymorphic followed by the Red Sokoto goat and then West African Dwarf. With respect to the nucleotide diversity, however, the Sahel goat was found to have the highest value followed by the West African Dwarf and the Red Sokoto goat. The high number of polymorphisms and haplotype diversity value in the Red Sokoto goat are not reflected in the nucleotide diversity because polymorphism in this breed is caused by only few frequent and many rare polymorphisms. The relatively high polymorphism of the Sahel goat at the *MC1R* locus is surprising because of the restricted distribution of this breed. The non-significant value of Tajima's D indicates no evidence for evolutionary selection (Suharyanto and Shiraishi, 2011).

The purpose of Tajima's D test is to distinguish between a DNA sequence evolving randomly ("neutrally") and one evolving under a non-random process, including directional selection or balancing selection, demographic expansion or contraction, genetic hitchhiking, or introgression. A randomly evolving DNA sequence contains mutations with no effect on the fitness and survival of an organism. The randomly evolving mutations are called "neutral", while mutations under selection are "non-neutral" (Tajima, 1989; Suharyanto and Shiraishi, 2011). For example, you would expect to find that a mutation which causes prenatal death or severe disease to be under selection. When looking at the human population as a whole, we say that the population frequency of a neutral mutation fluctuates randomly (i.e. the percentage of people in the population with the mutation changes from one generation to the next, and this

percentage is equally likely to go up or down, through genetic drift (Suharyanto and Shiraishi, 2011).

The strength of genetic drift depends on the population size. If a population is at a constant size with constant mutation rate, the population will reach an equilibrium of gene frequencies. The purpose of Tajima's test is to identify sequences which do not fit the neutral theory model at equilibrium between mutation and genetic drift (Tajima, 1989). Tajima's statistic computes a standardized measure of the total number of segregating sites (these are DNA sites that are polymorphic), with symbol S, in the sampled DNA and the average number of mutations between pairs in the sample, with symbol π. The two quantities whose values are compared are both method of moments estimates of the population genetic parameter theta, and so are expected to equal the same value. If these two numbers only differ by as much as one could reasonably expect by chance, then the null hypothesis of neutrality cannot be rejected. Otherwise, the null hypothesis of neutrality is rejected. But selection, demographic fluctuations and other violations of the neutral model (including rate heterogeneity and introgression) will change the expected values of S and π, so that they are no longer expected to be equal. The difference in the expectations for these two variables (which can be positive or negative) is the crux of Tajima's *D* test statistic. A positive value indicates an excess of intermediate frequency (polymorphic) alleles, while a negative value indicates an excess of rare alleles (Tajima, 1989; Suharyanto and Shiraishi, 2011).

The silent substitution *g.221T>C* was identified in both Nigerian and American goats and has also been reported by Fontanesi *et al.* (2009a) in six goat breeds, which include: Girgentana, Maltese, Derivata di Siria, Murciano-Granadina, Camosciata delle Alpi and Saanen. The fact that this mutation does not cause an amino acid change might be responsible for its presence in a number of goat breeds. The p.E55K mutation was also identified in both populations. Wu *et al.* (2006) also reported the p.K226E mutation in Boer goats.

Some of the *MC1R* mutations identified in this study are located in the first transmembrane domain. According to Schioth *et al.* (1997), most plasma membrane proteins contain an N-terminal signal peptide that targets the nascent protein to the endoplasmic reticulum (ER) membrane and is rapidly removed by proteolytic cleavage. However, the N-terminus of MC1R poorly matches the signal peptide consensus, and removal of the first 27 amino acids has no effect on ligand binding or expression levels in COS cells (Schioth *et al*., 1997). Moreover, a short Flag epitope fused to the MC1R N-terminus is not cleaved during the processing of the protein in HEK 293T (Sanchez-Mas *et al*., 2005) or MC1R-null B16G4F melanoma cells (Robinson and Healy, 2002). According to Wallin and von Heijne (1995), MC1R might belong to a class of membrane proteins that use the first TM domain as a non-cleavable signal anchor directing traffic of the protein. Consistent with the retention of the complete N-terminus in the mature protein, Chhajlani *et al.* (1996) found a mutation of Ser6 to Ala to cause a significant decrease of affinity for radiolabeled agonists. Mutations in the first transmembrane domain might, therefore, affect the trafficking of MC1R.

One of the mutations identified in the American goat breeds is located in the third intracellular loop. The intracellular loops of G-protein coupled receptors are known to provide the binding interfaces for the heterotrimeric G proteins and contain phosphorylation targets involved in the regulation of signaling, internalization and cycling. The third intracellular loop (IL3) is, however, poorly conserved in the melanocortin receptors (García-Borrón *et al*., 2005).

Another mutation identified in the American goat breeds is located in the sixth transmembrane domain. According to Yang *et al.* (1997) and García-Borrón *et al.* (2005), agonist binding is mostly accounted for by a network of charged and aromatic residues located in the transmembrane (TM) domains including the sixth transmembrane domain. A network of aromatic residues located near the extracellular side of TM4, 5 and 6 are known to contribute to agonist binding by interacting with the aromatic residues of the pharmacophore (Yang *et al*., 1997).

Some of the *MC1R* substitutions identified in this study occur at inserted positions relative to the Hidden Markov Model (HMM). According to Thomas *et al.* (2006), substitutions at inserted positions are not generally likely to be deleterious. The subPSEC and $P_{deleterious}$ scores for p.R229P were observed to be low which signifies a likely non-deleterious impact of the mutation. These low subPSEC and $P_{deleterious}$ values for the p.R229P mutation are not unexpected as the amino acid arginine is found not to be conserved across species.

The reason for the absence of the homozygous conditions for the p.E55K and p.N56K is not known. One hypothesis is that the mutated allele might be linked with a recessive lethal gene. This hypothesis, however, needs to be further investigated. The fact that the *g.221T>C* mutation identified in this study does not cause an amino acid change explains its relatively high frequency and presence in the homozygous condition in the West African Dwarf and Sahel breeds. Haplotype 1 seems to be the wild type haplotype due to its relatively high frequency in these breeds as well as other breeds of goat as seen from the studies of Fontanesi *et al.* (2009a).

The likely deleterious effect of the p.S52R and p.E55K mutations as seen from the *in silico* functional analysis using PANTHER might explain the absence of the homozygous conditions. The Alpine goats used in this study have the black and white coat colour. The p.E55K mutation was also found among the Nigerian goats. This mutation was found to be specific to black West African Dwarf goats. The p.E55K mutation might therefore contribute to the black coat colour seen in these breeds. The p.S52R which occurred with relatively high frequency in the Alpine breed might also contribute to the black coat colour seen in this breed. These mutations which occur in the first transmembrane domain probably cause permanent activation of the *MC1R* thereby resulting in black coat colour. According to Fontanesi *et al.* (2009a), permanent activations, caused by functional mutations, result in black coat colour whereas inactivating mutations cause red coat colour. According to Wu *et al.* (2006), a glutamic acid (E) to lysine (K) change is a gain of function mutation. This type of E →K substitution has been suggested to be

responsible for melanism in mice (Robins *et al.,* 1993), chickens (Kerje *et al.,* 2003) and birds (Mundy *et al.,* 2003).

The p.K226E mutation which was found to be specific to the Spanish breed studied was also identified by Wu *et al.* (2006) in Boer goats. Wu *et al.* (2006) suggested from their studies that this mutation is associated with the red or brown head phenotype in Boer goats. The Spanish breed and the Boer breed have a history of interbreeding (Browning *et al.,* 2004). This might, therefore, explain the presence of the p.K226E in the Spanish breed. According to Wu *et al.* (2006),"there are four conservative Ks at the cytoplasmic domain in goat *MC1R* protein sequences; consequently, the conservative 226K at the third cytoplasmic domain may play an important role in determining the function of MC1R." The K226E mutation is a replacement of a positive charged K with a negative charged E. According to them, this kind of K→E mutation may be a loss of function mutation, resulting in the loss of melanin production. This might therefore lead to red coat colour.

5.3 DNA POLYMORPHISMS AND HAPLOTYPE PATTERNS OF THE *AGOUTI SIGNALING PROTEIN* (*ASIP)* GENE

Both nucleotide and predicted amino acid sequences of the *ASIP* gene fragment amplified in this study showed 99% and 98% similarity, respectively, using the Basic Local Alignment Search Tool (BLAST) from www.pubmed.org, to the caprine *MC1R* sequences in the EMBL database with accession numbers AM746057.2 and CAN88844.2, respectively (Appendices 7 and 8).

The level of DNA polymorphism observed in the portion of *ASIP* gene studied within the Nigerian goat breeds is strikingly high. It is much higher than for most animal genes (Moriyama and Powell, 1996). According to Fontanesi *et al.* (2009b), more than 50 alleles have been observed at the a*gouti* locus in mice with most having been characterized at the DNA level. Similar values were not obtained for the American breeds. This is probably due to the small sample size. The negative Tajima's D values recorded for the Nigerian goats, however, indicates an excess of rare alleles (Tajima, 1989; Suharyanto and Shiraishi, 2011). The high level of DNA polymorphism in *ASIP* within the Nigerian goat breeds, which is reflected in the number of polymorphism and Tajima's D values, could be due to the action of balancing selection in or near the locus. Theory (Strobeck 1983; Hudson and Kaplan 1988; Kaplan *et al.* 1988) suggests that there should be a peak of polymorphism and linkage disequilibrium near a site that is under balancing selection. Significant negative values were obtained for Tajima's D which is indicative of negative or purifying selection against genotypes carrying the less frequent alleles (Suharyanto and Shiraishi, 2011). Despite the fact that the American breeds of goat studied have been selected for coat colour, non-significant Tajima's D values were recorded. This indicates probable lack of association between polymorphisms within the region of the *ASIP* locus studied and coat colour in these breeds.

The low and high levels of nucleotide as well as haplotype diversites of the Sahel and Red Sokoto goats, respectively, are probably due to their respective restricted and wide distribution. The Sahel goat is restricted to the Northern border of Nigeria, particularly in Borno State while the Red Sokoto goat is the most common breed of goat in Nigeria. It is

found majorly in the NorthWest, NorthEast, and NorthCentral and even in the South (Blench, 1999).

The *g.293G>A* mutation was found to be common to both the American and Nigerian populations. Although this is an intronic mutation, such mutations have been found to alter gene expression by affecting regulation, impairing protein synthesis and causing aberrant splicing (Miné *et al.,* 2003; Holla *et al.,* 2009; Nascimbeni *et al.,* 2010). For example, a splice mutation in intron 17 of the *KIT* gene (*Dominant white* locus), affecting coat colour has been identified in several pig breeds (Marklund *et al*., 1998; Pielberg *et al*., 2002).

The caprine ASIP protein sequence contains 133 amino acids with a characteristic signature for mammalian ASIP protein. A signal sequence of 22 amino acids is followed by a basic amino-terminal domain of 65 residues (containing 19 lysines or arginines), 4 prolines, and a 42-residue cysteine-rich carboxy-terminal domain, whose pattern of spacing is highly conserved among vertebrates (McNulty *et al.,* 2004; Fontanesi *et al.,* 2009b). The carboxyl-terminal domain was reported by McNulty *et al.* (2004) to be the main domain responsible for melanocortin receptor binding activity in vertebrates while the basic amino-terminal domain, according to Jackson *et al.* (2006), is required for interaction with Attractin, a large single-transmembrane-spanning domain protein that is required for ASIP signaling *in vivo*, and is thought to act as an accessory receptor for ASIP-mediated antagonism of MC1R. In this study, lysines at positions 36 and 45 were found to be in highly conserved positions of the mammalian ASIP basic amino-terminal

domain. The lysine residues might be highly important since they make up the 19 lysines or arginine found in the basic amino-terminal domain. *In silico* functional analysis using PANTHER also revealed relatively high subSPEC and $P_{deleterious}$ values for the p.L36V and p.L45W mutations indicating a likely deleterious effect of these mutations while the p.N35K, asparagine, a polar (hydrophilic) amino acid, is substituted with lysine, a positively charged amino acid, the p.M42L, methionine, a non-polar (hydrophobic) amino acid, is substituted with leucine, another non-polar amino acid. These mutations might not have functional impact on the protein as predicted by PANTHER. Serine at position 19 is found only in goat, as seen in this study and that of Fontanesi *et al.* (2009b), and sheep (accession number NP_001127775.1). Serine and threonine are polar (hydrophilic) residues while arginine is a positively charged amino acid. This might explain why serine is substituted with threonine in a number of species. Substitution of serine with arginine as seen in this study might lead to a reduction in translocation of the ASIP protein since signal sequences are known to mediate the targeting of nascent secreted proteins to membranes (Schioth *et al.,* 1997). In a study by Arnold *et al.* (1990), a T to C point mutation changing the codon at position 18 of the 31 amino acid prepro sequence of preproparathyroid hormone from cysteine (a hydrophilic amino acid) to arginine in one familial isolated hypoparathyroidism (FIH) kindred caused a functional defect. The mutation was found to cause a disruption of the core that leads into impaired interaction of the nascent protein with the signal recognition particle, the translocation machinery, and signal peptidase cleavage. A *g.279G>A* mutation, causing a Thr (ACG) to Ala (GCG) substitution in the C-terminus of the signal peptide in preprovasopressin (preproVP) has also been identified in patients with familial central diabetes insipidus

(Ito *et al.*, 1993). According to Ito *et al.* (1993), inefficient processing of preproVP produced by a mutant allele is possibly involved in the pathogenesis of diabetes insipidus in the affected individuals. *In silico* functional analysis of the p.S19R mutation using PANTHER, however, indicates a likely non-deleterious effect of this missense mutation in goats.

There seems to be no association between the twenty *ASIP* haplotypes found among the Nigerian goats and coat colour in this study. A large number of alleles have also been reported at the *ASIP* locus from previous studies. These alleles have, however, been found to have effects on coat colour (Searle, 1968; Dickie, 1969; Adalsteinsson *et al*., 1994). At the *ASIP* locus in mice for instance, more than 50 alleles have been observed with most having been characterized at the DNA level (e.g., Bultman *et al.*, 1992; Miltenberger *et al*., 2002). Mutations at this locus affecting or associated with coat colours or skin pigmentation have also been identified in other species. These mutations have been found to affect both non coding (human: Kanetsky *et al*., 2002; cattle: Girardot *et a*l., 2006) and coding regions (rat, Kuramoto *et al*., 2001; horse, Rieder *et al*., 2001; dog, Berryere *et al*., 2005; Kerns *et al*., 2004; cat, Eizirik *et al.*, 2003; fox, Våge *et al*., 1997). In sheep, regulatory mutations in the *ASIP* gene were suggested to cause the nonagouti recessive black coat colour (Norris and Whan, 2008; Royo *et al*., 2008).

The *g.293G>A* and *g.327C>A* mutations found among the American goats might contribute to the black coat colour observed in the Alpine breed. More animals, however, need to be sampled to draw reasonable conclusions. Fontanesi *et al.* (2009b) also sequenced the coding region of the goat *ASIP* gene in 6 goat breeds (Girgentana, Maltese,

Derivata di Siria, Murciano-Granadina, Camosciata delle Alpi, and Saanen), with different coat colours and patterns. Five single nucleotide polymorphisms (SNPs) were identified from their studies, 3 of which caused missense mutations in conserved positions of the cysteine-rich carboxy-terminal domain of the protein (p.Ala96Gly, p.Cys126Gly, and p.Val128Gly). Allele and genotype frequencies from their studies, however, suggested that these mutations are not associated or not completely associated with coat colour in the investigated goat breeds

The results from these studies on *MC1R* and *ASIP* genes seem to support the hypothesis of epistatic interaction between *MC1R* and *ASIP*, i.e., the possibility to express the *ASIP* alleles can be obtained only when at least a copy of a putative wild type allele at the *extension* locus is present. The white phenotype in the Saanen and Sahel goats maybe due to the presence of the wild type (A^{wt}) allele at the *ASIP* locus as described in other goat breeds with white coat colour (Adalsteinsson *et al*., 1994; Sponenberg *et al*., 1998). About 82.4% of the Sahel goats investigated in this study belong to the wild type *MC1R* haplotype. The remaining 17.6% belong to haplotype 2 which carries the silent *g.221T>C* mutation. All the Saanen goats belong to the wild type *MC1R* haplotype. All the Saanen and Sahel goats investigated in this study also belong to the wild type *ASIP* haplotype. Fontanesi *et al.* (2009b) also reported the association of the A^{wt} allele with white coat colour in the Saanen breed. The biochemical mechanism underlying the development of the white coat colour is, however, not yet clear. According to Fontanesi *et al.* (2009b), gain of function mutations produce black/dark coat colour, whereas loss of function mutations cause red/yellow or white coat colour. One can therefore hypothesize that the

A^{wt} allele results in a functional agouti signaling protein which prevents the binding of αMSH to MC1R. This therefore causes a loss of function of the MC1R, possibly leading to the white coat colour.

There seems to be no clear association between red coat colour phenotype in the Red Sokoto goats and mutations at the *MC1R* and *ASIP* loci in this study. All the Red Sokoto goats belong to the wild type *MC1R* haplotype and 89.1% of them belong to the wild type *ASIP* haplotype. Fontanesi *et al.* (2009a) also observed a surprising incomplete association of the nonsense mutation p.Q225X at the *MC1R* locus with red coat colour in Derivata di Siria goats. This mutation is expected to produce a protein that lacks the 93 C-terminal amino acids (including part of the third intracellular loop, the last two transmembrane domains, the last intercellular loop, and the intracellular tail) and for this reason the receptor may not be functional. According to the solid red coat colour of the Derivata di Siria breed, one would expect the fixation of the nonsense allele that should cause the production of a non-functional transmembrane receptor, as reported in several other species for similar disrupting mutations (Robbins *et al.,* 1993; Joerg *et al.,* 1996; Everts *et al.,* 2000; Newton *et al.,* 2000; Fontanesi *et al.,* 2006). Fontanesi *et al.* (2009b) also found no association between red coat colour and mutations in the *ASIP* gene.

Mutations at the *MC1R* and *ASIP* loci may determine eumelanic and pheomelanic phenotypes in these goat breeds. They are, however, probably not the only factors. Other genes might be involved in determining red coat colour especially. A number of possible upstream or downstream processes, such as *β-defensin* and *proopiomelanocortin* (*POMC*), might be involved in the production of red and black coat colour phenotypes.

Recently, a β-defensin protein has been shown to be a ligand of MC1R and competitor of the ASIP protein. Mutations in this gene cause black or brindle coat colour in dog (Candille *et al.,* 2007). Also, sequential cleavage of the coded precursor protein of *POMC* produces the αMSH that binds MC1R and competes with ASIP in regulating, in turn, melanin synthesis. Mutations in the *POMC* gene have been shown to cause red hair pigmentation in humans (Krude *et al.,* 1998).

5.4 VARIATION OF g.*469*C>G IN THE MELANOPHILIN (*MLPH*) GENE INTRON 2 IN NIGERIAN GOAT BREEDS

Although the transversion *g.469C>G* is an intronic mutation, its association with coat colour cannot be overlooked as it has been found that mutations in intronic regions can alter gene expression by affecting regulation, impairing protein synthesis and causing aberrant splicing (Miné *et al.,* 2003; Holla *et al.,* 2009; Nascimbeni *et al.,* 2010). The positive value of Wright's fixation index observed within the Red Sokoto breed is probably due to its distribution. The Red Sokoto breed is the most common and most widespread breed of goat in Nigeria. Its use for high quality leather has had important consequences on the distribution of this breed (Blench, 1999). The relatively low degree of genetic variation for the Sahel breed can be attributed to its restricted geographical distribution. This breed is found mostly along the northern border of Nigeria, particularly in Borno State (Blench, 1999). The relatively low genetic diversity of the three Nigerian goat breeds at the *g.469C>G* locus might be due to the directional selection for coat colour. The Veterinary Service in Sokoto Province was once said to have castrated

219,688 non-red male goats in 5 years to replace the non-red skins with the more valuable red in the local markets (Blench, 1999). The overall low Fis and Fst values and high Nm value observed in this study shows that there is little or no differentiation within and among the breeds based on *g.469C>G.* The result of the genetic identity between the Nigerian goat breeds at the *g.469C>G* locus is consistent with the result on the effect of breed on morphological traits where a non-significant difference was reported between Red Sokoto and Sahel for most of the body dimensions and significant differences between Red Sokoto and West African Dwarf, and Sahel and West African Dwarf goats were observed for most of the body dimensions.

Both West African Dwarf and Sahel goats were found to be in Hardy-Weinberg equilibrium at 0.05 rejection level for g.*469C>G*. Overall, the population of Nigerian goats was also found to be in Hardy-Weinberg equilibrium with respect to this locus ($P<0.05$). The Hardy-Weinberg principle states that in a large randomly breeding population, allelic frequencies will remain the same from generation to generation assuming that there is no mutation, gene migration, selection or genetic drift. The non-significant P value for the Hardy–Weinberg equilibrium test observed for the overall population and for the West African Dwarf and Sahel goats probably depicts that the Nigerian goats are not under selection with regard to this locus. The significant P value for the Red Sokoto goats depicts either selection or reproductive isolation. Reproductive isolation is, however, most probable since the Red Sokoto goat has a wide distribution and this, very likely, results in inbreeding. From this study, the g.*469C>G* mutation did not also seem to be associated with coat colour based on the allele and genotypic

frequencies in the different breeds. Zhou *et al.* (2010), however, found an association between coat colour and the missense mutation g.*11584A>G* found in exon 10 of caprine *MLPH* gene. One of the alleles, allele G, was mostly found in Chengdu Ma goat and Nanjiang Brown goat (including three strains), in which homozygote GG was only found. They inferred that allele G might be a candidate site for the dilute coat colour (tan) found in Nanjiang Brown goat and Chengdu Ma goat. Li *et al.* (2010) also found an association between nine completely linked SNPs and dilute coat colour (tan) of Chengdu Ma goat. The g.*469C>G* mutation was, however, not reported in either of these studies.

5.5 ASSOCIATION BETWEEN IDENTIFIED MUTATIONS AT THE *MC1R* LOCUS AND SOME BODY TRAITS IN NIGERIAN GOATS

Lack of association between genotypes at the *MC1R* locus and morphological traits was observed in this study. This observation is contrary to the report on the effect of coat colour on some morphological traits in Nigerian goats, where an effect on body length, body weight and heart girth was observed, in this research. This is also contrary to the report of McLean and Schmutz (2009) where an association between *MC1R* genotype and growth and carcass traits in beef cattle was observed. They reported that black cattle of E^D/E^D or E^D/e genotype had increased back fat and required significantly fewer days (15-25) on feed to reach a target fat level for slaughter than the red cattle. They also observed that red cattle of e/e genotype had a significantly larger longissimus dorsi (l. dorsi), shipping weight and hot carcass weight. They speculated that the differences observed in weight of the cattle were related to different α-MSH binding to MC4R,

among cattle of different MC1R genotypes. According to them, although α-MSH binds to MC1R, MC3R, MC4R and MC5R, the mutation in MC1R of red cattle prevents binding. Therefore black cattle with an $E^{D}/\_$ genotype would gain weight and deposit fat more readily than e/e animals due to more α-MSH binding to MC1R. Cattle of e/e genotype (red to cream colour) would direct more α-MSH to the MC4R pathway reducing overall appetite, taking longer to finish and deposit fat. Mutations in other species that cause a decrease in the production of the peptide in either *POMC*, the gene that encodes α-MSH, or in MC4R, have been found to affect fat deposition in a gene dosage effect manner (Cone, 2005). Another hypothesis from their studies is that the MC1R mutation causing red coat colour is in linkage disequilibrium with another mutation in another gene that causes weight and fat deposition differences in cattle.

The lack of association between the *MC1R* genotypes and body traits in this study is probably due to the small sample size. It will be necessary to involve more animals in order to have sufficient statistical power, i.e. a good chance of detecting a statistically significant result.

6.0 SUMMARY OF FINDINGS/CONCLUSIONS

1. The results of this research showed that coat colour significantly ($P<0.05$) affects body weight, body length and heart girth in Nigerian goats.

2. All the measurements except rump width and ear length were significantly ($P<0.05$) affected by breed type.

3. Sex influenced ($P<0.05$) only heart girth while age influenced ($P<0.05$) all but tail length.

4. The p.E55K and p.N56K mutations identified at the *MC1R* locus in Nigerian goats were found to be specific to some black West African Dwarf goats and might be associated with black coat colour.

5. In the American breeds, p.S52R and p.E55K were found to be specific to the Alpine breed (black and white). The p.E55K is common to both Nigerian and American goats and was found to be specific to some black West African Dwarf and Alpine breed (black and white). This mutation might therefore be associated with black coat colour.

6. The p.K226E and p.R229P mutations were found to be specific to the Spanish breed (brown and white). The p.K226E mutation has been identified to be responsible for the red neck in Boer goats from previous studies. It is not surprising to find this mutation in Spanish goats since both breeds are usually crossed for improved meat quality.

7. About 82.4% of the Sahel goats investigated in this study belong to the wild type *MC1R* haplotype. The remaining 17.6% belong to haplotype 2 which carries the silent *g.221T>C* mutation. All the Saanen goats belong to the wild type *MC1R* haplotype. All the Saanen and Sahel goats investigated in this study also belong to the wild type *ASIP* haplotype. The white phenotype in the Saanen and Sahel goats is, therefore, very likely to be a result of an epistatic interaction between *MC1R* and *ASIP* such that the possibility to express the *ASIP* alleles can be obtained only when at least a copy of a putative wild type allele at the *MC1R* locus is present.

8. The *g.293G>A* and *g.327C>A* mutations found in the American breeds seem to be specific to the Alpine breed. These mutations might contribute to the black colour observed in this breed.

9. No significant differences were found to exist between Nigerian breeds for the *g.469C>G* mutation at the *MLPH* locus.

10. Overall, it appears that the populations of Nigerian goats is in Hardy-Weinberg equilibrium with respect to the *g.469C>G* mutation at the *MLPH* locus.

11. Lack of association between genotypes at the *MC1R* locus and morphological traits was observed in this study.

7.0 CONTRIBUTIONS TO KNOWLEDGE

1. New mutations in *MC1R, ASIP* and *MLPH* genes were identified in this study and some of the identified mutations were found to be specific to the black coat colour.
2. From the results of this study, an epistatic interaction between *MC1R* and *ASIP* was proposed to produce the white coat colour in both Nigerian and American goats. The mechanism underlying the development of the white coat colour was thus hypothesized.
3. Mutations in the *MC1R, ASIP* and *MLPH* genes did not seem to be associated with red coat colour in goat from this study. Other mammalian coat colour genes are therefore proposed for further research.

8.0 IMPLICATION FOR FURTHER STUDY/RECOMMENDATION

1. Other mammalian coat colour genes are proposed for further research since mutations in the *MC1R*, *ASIP* and *MLPH* genes did not seem to be associated with red coat colour.

2. The result shows that, as with the American and other breeds, breeding programmes can be designed around coat colours and body traits such as body weight, body length and heart girth which seem to have a selective value in the different parts of Nigeria.

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Web pages

http://goatkingdom.tripod.com/meat-goat-production

APPENDIX

Appendix 1: Pairwise comparisons between coat colours

Pairwise Comparisons

Dependent Variable	(I) coatcolour	(J) coatcolour	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c]	
						Lower Bound	Upper Bound
bodyweight	1.00	2.00	-1.796[a,b]	1.905	.346	-5.544	1.952
		3.00	-7.004[a,b,*]	1.648	.000	-10.246	-3.761
		4.00	-3.893[a,b,*]	1.619	.017	-7.079	-.707
	2.00	1.00	1.796[a,b]	1.905	.346	-1.952	5.544
		3.00	-5.207[a,b,*]	1.527	.001	-8.212	-2.203
		4.00	-2.097[a,b]	1.496	.162	-5.040	.847
	3.00	1.00	7.004[a,b,*]	1.648	.000	3.761	10.246
		2.00	5.207[a,b,*]	1.527	.001	2.203	8.212
		4.00	3.111[a,b,*]	1.151	.007	.846	5.376
	4.00	1.00	3.893[a,b,*]	1.619	.017	.707	7.079
		2.00	2.097[a,b]	1.496	.162	-.847	5.040
		3.00	-3.111[a,b,*]	1.151	.007	-5.376	-.846
bodylength	1.00	2.00	-2.868[a,b]	3.171	.366	-9.107	3.371
		3.00	-7.542[a,b,*]	2.743	.006	-12.939	-2.145
		4.00	-.707[a,b]	2.695	.793	-6.011	4.596
	2.00	1.00	2.868[a,b]	3.171	.366	-3.371	9.107
		3.00	-4.674[a,b]	2.541	.067	-9.675	.327
		4.00	2.161[a,b]	2.490	.386	-2.739	7.061
	3.00	1.00	7.542[a,b,*]	2.743	.006	2.145	12.939
		2.00	4.674[a,b]	2.541	.067	-.327	9.675
		4.00	6.835[a,b,*]	1.916	.000	3.065	10.605
	4.00	1.00	.707[a,b]	2.695	.793	-4.596	6.011
		2.00	-2.161[a,b]	2.490	.386	-7.061	2.739

		3.00	-6.835[a,b,*]	1.916	.000	-10.605	-3.065
taillength	1.00	2.00	.827[a,b]	.541	.127	-.237	1.891
		3.00	-.302[a,b]	.468	.519	-1.223	.618
		4.00	-.098[a,b]	.460	.831	-1.003	.806
	2.00	1.00	-.827[a,b]	.541	.127	-1.891	.237
		3.00	-1.129[a,b,*]	.433	.010	-1.982	-.276
		4.00	-.925[a,b,*]	.425	.030	-1.761	-.090
	3.00	1.00	.302[a,b]	.468	.519	-.618	1.223
		2.00	1.129[a,b,*]	.433	.010	.276	1.982
		4.00	.204[a,b]	.327	.533	-.439	.847
	4.00	1.00	.098[a,b]	.460	.831	-.806	1.003
		2.00	.925[a,b,*]	.425	.030	.090	1.761
		3.00	-.204[a,b]	.327	.533	-.847	.439
withersheight	1.00	2.00	-2.490[a,b]	1.814	.171	-6.061	1.080
		3.00	-6.428[a,b,*]	1.570	.000	-9.516	-3.339
		4.00	-5.194[a,b,*]	1.542	.001	-8.229	-2.158
	2.00	1.00	2.490[a,b]	1.814	.171	-1.080	6.061
		3.00	-3.937[a,b,*]	1.454	.007	-6.799	-1.075
		4.00	-2.703[a,b]	1.425	.059	-5.507	.101
	3.00	1.00	6.428[a,b,*]	1.570	.000	3.339	9.516
		2.00	3.937[a,b,*]	1.454	.007	1.075	6.799
		4.00	1.234[a,b]	1.096	.261	-.924	3.392
	4.00	1.00	5.194[a,b,*]	1.542	.001	2.158	8.229
		2.00	2.703[a,b]	1.425	.059	-.101	5.507
		3.00	-1.234[a,b]	1.096	.261	-3.392	.924
rumpheight	1.00	2.00	-4.203[a,b]	2.217	.059	-8.566	.159
		3.00	-5.350[a,b,*]	1.918	.006	-9.124	-1.576
		4.00	-4.742[a,b,*]	1.885	.012	-8.450	-1.033
	2.00	1.00	4.203[a,b]	2.217	.059	-.159	8.566
		3.00	-1.147[a,b]	1.777	.519	-4.643	2.350
		4.00	-.538[a,b]	1.741	.757	-3.964	2.888

	3.00	1.00	5.350[a,b,*]	1.918	.006	1.576	9.124
		2.00	1.147[a,b]	1.777	.519	-2.350	4.643
		4.00	.608[a,b]	1.340	.650	-2.028	3.244
	4.00	1.00	4.742[a,b,*]	1.885	.012	1.033	8.450
		2.00	.538[a,b]	1.741	.757	-2.888	3.964
		3.00	-.608[a,b]	1.340	.650	-3.244	2.028
heartgirth	1.00	2.00	-.852[a,b]	2.465	.730	-5.703	3.999
		3.00	-6.476[a,b,*]	2.133	.003	-10.673	-2.279
		4.00	-1.083[a,b]	2.096	.606	-5.206	3.041
	2.00	1.00	.852[a,b]	2.465	.730	-3.999	5.703
		3.00	-5.624[a,b,*]	1.976	.005	-9.512	-1.735
		4.00	-.230[a,b]	1.936	.905	-4.040	3.580
	3.00	1.00	6.476[a,b,*]	2.133	.003	2.279	10.673
		2.00	5.624[a,b,*]	1.976	.005	1.735	9.512
		4.00	5.393[a,b,*]	1.490	.000	2.462	8.325
	4.00	1.00	1.083[a,b]	2.096	.606	-3.041	5.206
		2.00	.230[a,b]	1.936	.905	-3.580	4.040
		3.00	-5.393[a,b,*]	1.490	.000	-8.325	-2.462
chestdepth	1.00	2.00	-1.492[a,b]	1.159	.199	-3.773	.789
		3.00	-2.523[a,b,*]	1.003	.012	-4.496	-.550
		4.00	-1.506[a,b]	.985	.127	-3.445	.433
	2.00	1.00	1.492[a,b]	1.159	.199	-.789	3.773
		3.00	-1.031[a,b]	.929	.268	-2.859	.798
		4.00	-.014[a,b]	.910	.988	-1.806	1.777
	3.00	1.00	2.523[a,b,*]	1.003	.012	.550	4.496
		2.00	1.031[a,b]	.929	.268	-.798	2.859
		4.00	1.017[a,b]	.700	.148	-.362	2.395
	4.00	1.00	1.506[a,b]	.985	.127	-.433	3.445
		2.00	.014[a,b]	.910	.988	-1.777	1.806
		3.00	-1.017[a,b]	.700	.148	-2.395	.362
rumpwidth	1.00	2.00	-.918[a,b]	.563	.104	-2.025	.190

		3.00	-1.102[a,b,*]	.487	.024	-2.059	-.144
		4.00	-.808[a,b]	.478	.092	-1.750	.133
	2.00	1.00	.918[a,b]	.563	.104	-.190	2.025
		3.00	-.184[a,b]	.451	.684	-1.072	.704
		4.00	.109[a,b]	.442	.805	-.760	.979
	3.00	1.00	1.102[a,b,*]	.487	.024	.144	2.059
		2.00	.184[a,b]	.451	.684	-.704	1.072
		4.00	.293[a,b]	.340	.389	-.376	.962
	4.00	1.00	.808[a,b]	.478	.092	-.133	1.750
		2.00	-.109[a,b]	.442	.805	-.979	.760
		3.00	-.293[a,b]	.340	.389	-.962	.376
forcanonbnlnt	1.00	2.00	-1.102[a,b,*]	.530	.038	-2.145	-.059
		3.00	-1.210[a,b,*]	.459	.009	-2.113	-.308
		4.00	-1.579[a,b,*]	.451	.001	-2.466	-.693
	2.00	1.00	1.102[a,b,*]	.530	.038	.059	2.145
		3.00	-.108[a,b]	.425	.799	-.945	.728
		4.00	-.477[a,b]	.416	.252	-1.297	.342
	3.00	1.00	1.210[a,b,*]	.459	.009	.308	2.113
		2.00	.108[a,b]	.425	.799	-.728	.945
		4.00	-.369[a,b]	.320	.250	-.999	.261
	4.00	1.00	1.579[a,b,*]	.451	.001	.693	2.466
		2.00	.477[a,b]	.416	.252	-.342	1.297
		3.00	.369[a,b]	.320	.250	-.261	.999
earlength	1.00	2.00	-.147[a,b]	.476	.758	-1.083	.789
		3.00	-.356[a,b]	.411	.387	-1.166	.453
		4.00	-.551[a,b]	.404	.174	-1.347	.244
	2.00	1.00	.147[a,b]	.476	.758	-.789	1.083
		3.00	-.209[a,b]	.381	.583	-.960	.541
		4.00	-.405[a,b]	.373	.279	-1.139	.330
	3.00	1.00	.356[a,b]	.411	.387	-.453	1.166
		2.00	.209[a,b]	.381	.583	-.541	.960

		4.00	-.195[a,b]	.287	.498	-.761	.370
	4.00	1.00	.551[a,b]	.404	.174	-.244	1.347
		2.00	.405[a,b]	.373	.279	-.330	1.139
		3.00	.195[a,b]	.287	.498	-.370	.761

Based on estimated marginal means

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

c. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

*. The mean difference is significant at the .05 level.

Black is coded as 1, brown as 2, red as 3 and white as 4

Appendix 2: Pairwise comparisons between breeds

Pairwise Comparisons

Dependent Variable	(I) breed	(J) breed	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c]	
						Lower Bound	Upper Bound
bodyweight	red	sahel	-2.859*,a,b	1.394	.041	-5.603	-.115
		wad	2.574*,a,b	1.188	.031	.237	4.911
	sahel	red	2.859*,a,b	1.394	.041	.115	5.603
		wad	5.433*,a,b	1.183	.000	3.105	7.760
	wad	red	-2.574*,a,b	1.188	.031	-4.911	-.237
		sahel	-5.433*,a,b	1.183	.000	-7.760	-3.105
bodylength	red	sahel	-1.358,a,b	2.321	.559	-5.925	3.210
		wad	9.845*,a,b	1.977	.000	5.954	13.735
	sahel	red	1.358,a,b	2.321	.559	-3.210	5.925
		wad	11.202*,a,b	1.969	.000	7.328	15.076
	wad	red	-9.845*,a,b	1.977	.000	-13.735	-5.954
		sahel	-11.202*,a,b	1.969	.000	-15.076	-7.328
taillength	red	sahel	-.331,a,b	.396	.403	-1.110	.448
		wad	1.314*,a,b	.337	.000	.651	1.978
	sahel	red	.331,a,b	.396	.403	-.448	1.110
		wad	1.645*,a,b	.336	.000	.985	2.306
	wad	red	-1.314*,a,b	.337	.000	-1.978	-.651
		sahel	-1.645*,a,b	.336	.000	-2.306	-.985
dewithersheight	red	sahel	-2.364,a,b	1.328	.076	-4.978	.249
		wad	11.997*,a,b	1.131	.000	9.770	14.223
	sahel	red	2.364,a,b	1.328	.076	-.249	4.978
		wad	14.361*,a,b	1.127	.000	12.144	16.578
	wad	red	-11.997*,a,b	1.131	.000	-14.223	-9.770
		sahel	-14.361*,a,b	1.127	.000	-16.578	-12.144
rumpheight	red	sahel	-1.603,a,b	1.623	.324	-4.796	1.591
		wad	11.829*,a,b	1.382	.000	9.109	14.549

	sahel	red	1.603[a,b]	1.623	.324	-1.591	4.796
		wad	13.432*[a,b]	1.377	.000	10.723	16.141
	wad	red	-11.829*[a,b]	1.382	.000	-14.549	-9.109
		sahel	-13.432*[a,b]	1.377	.000	-16.141	-10.723
heartgirth	red	sahel	-6.007*[a,b]	1.805	.001	-9.558	-2.456
		wad	1.729[a,b]	1.537	.262	-1.296	4.754
	sahel	red	6.007*[a,b]	1.805	.001	2.456	9.558
		wad	7.736*[a,b]	1.531	.000	4.724	10.748
	wad	red	-1.729[a,b]	1.537	.262	-4.754	1.296
		sahel	-7.736*[a,b]	1.531	.000	-10.748	-4.724
chestdepth	red	sahel	-.895[a,b]	.849	.292	-2.565	.775
		wad	3.603*[a,b]	.723	.000	2.181	5.026
	sahel	red	.895[a,b]	.849	.292	-.775	2.565
		wad	4.498*[a,b]	.720	.000	3.081	5.914
	wad	red	-3.603*[a,b]	.723	.000	-5.026	-2.181
		sahel	-4.498*[a,b]	.720	.000	-5.914	-3.081
rumpwidth	red	sahel	-.326[a,b]	.412	.430	-1.136	.485
		wad	.730*[a,b]	.351	.038	.039	1.420
	sahel	red	.326[a,b]	.412	.430	-.485	1.136
		wad	1.055*[a,b]	.349	.003	.368	1.743
	wad	red	-.730*[a,b]	.351	.038	-1.420	-.039
		sahel	-1.055*[a,b]	.349	.003	-1.743	-.368
forcanonbnlnt	red	sahel	-.586[a,b]	.388	.132	-1.349	.178
		wad	3.045*[a,b]	.331	.000	2.395	3.696
	sahel	red	.586[a,b]	.388	.132	-.178	1.349
		wad	3.631*[a,b]	.329	.000	2.983	4.279
	wad	red	-3.045*[a,b]	.331	.000	-3.696	-2.395
		sahel	-3.631*[a,b]	.329	.000	-4.279	-2.983
earlength	red	sahel	-.170[a,b]	.348	.626	-.855	.515
		wad	.560[a,b]	.297	.060	-.024	1.143
	sahel	red	.170[a,b]	.348	.626	-.515	.855

		wad	.730*,a,b	.295	.014	.149	1.311
	wad	red	-.560',a,b	.297	.060	-1.143	.024
		sahel	-.730*,a,b	.295	.014	-1.311	-.149

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

c. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Appendix 3: Pairwise comparisons between age groups

Estimates

Dependent Variable	age	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
bodyweight	1.00	17.208[a]	.839	15.557	18.859
	2.00	19.985[a]	.849	18.314	21.657
	3.00	24.645[a]	.942	22.791	26.499
bodylength	1.00	71.404[a]	1.396	68.656	74.152
	2.00	76.971[a]	1.414	74.189	79.754
	3.00	83.604[a]	1.568	80.517	86.690
taillength	1.00	11.155[a]	.238	10.687	11.624
	2.00	11.262[a]	.241	10.787	11.737
	3.00	11.775[a]	.267	11.249	12.302
withersheight	1.00	47.472[a]	.799	45.900	49.044
	2.00	51.589[a]	.809	49.997	53.181
	3.00	55.082[a]	.898	53.316	56.848
rumpheight	1.00	48.392[a]	.976	46.470	50.313
	2.00	53.468[a]	.989	51.522	55.413
	3.00	58.873[a]	1.097	56.715	61.031
heartgirth	1.00	58.632[a]	1.086	56.496	60.769
	2.00	62.658[a]	1.099	60.495	64.822
	3.00	67.513[a]	1.219	65.113	69.912
chestdepth	1.00	22.823[a]	.511	21.819	23.828
	2.00	24.621[a]	.517	23.604	25.639
	3.00	26.874[a]	.573	25.746	28.002
rumpwidth	1.00	9.467[a]	.248	8.979	9.955
	2.00	10.638[a]	.251	10.144	11.131
	3.00	11.904[a]	.278	11.357	12.452
forcanonbnlnt	1.00	11.238[a]	.233	10.779	11.698

	2.00	12.093[a]	.236	11.628	12.558
	3.00	13.177[a]	.262	12.661	13.693
earlength	1.00	10.562[a]	.209	10.150	10.974
	2.00	10.839[a]	.212	10.422	11.257
	3.00	11.752[a]	.235	11.290	12.215

a. Based on modified population marginal mean.

1-2 years is coded as 1, 2-3 years is coded as 2, and 3-4 years is coded as 3

Appendix 4: Pairwise comparisons between sexes

Pairwise Comparisons

Dependent Variable	(I) sex	(J) sex	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c]	
						Lower Bound	Upper Bound
bodyweight	1.00	2.00	-2.153*,a,b	1.041	.039	-4.202	-.105
	2.00	1.00	2.153*,a,b	1.041	.039	.105	4.202
bodylength	1.00	2.00	-2.417,a,b	1.733	.164	-5.827	.992
	2.00	1.00	2.417,a,b	1.733	.164	-.992	5.827
taillength	1.00	2.00	.592*,a,b	.295	.046	.011	1.174
	2.00	1.00	-.592*,a,b	.295	.046	-1.174	-.011
withersheight	1.00	2.00	.763,a,b	.992	.442	-1.188	2.714
	2.00	1.00	-.763,a,b	.992	.442	-2.714	1.188
rumpheight	1.00	2.00	2.109,a,b	1.211	.083	-.275	4.493
	2.00	1.00	-2.109,a,b	1.211	.083	-4.493	.275
heartgirth	1.00	2.00	-4.572*,a,b	1.347	.001	-7.223	-1.922
	2.00	1.00	4.572*,a,b	1.347	.001	1.922	7.223
chestdepth	1.00	2.00	-.702,a,b	.633	.269	-1.948	.545
	2.00	1.00	.702,a,b	.633	.269	-.545	1.948
rumpwidth	1.00	2.00	-.456,a,b	.307	.139	-1.061	.149
	2.00	1.00	.456,a,b	.307	.139	-.149	1.061
forcanonbnlnt	1.00	2.00	.772*,a,b	.290	.008	.202	1.342
	2.00	1.00	-.772*,a,b	.290	.008	-1.342	-.202
earlength	1.00	2.00	.152,a,b	.260	.559	-.359	.664
	2.00	1.00	-.152,a,b	.260	.559	-.664	.359

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

c. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Male is coded as 1, Female as 2

Appendix 5: Basic Local Alignment Search Tool (BLAST) output of the *MC1R* nucleotide sequence

>emb|FM212940.1| Capra hircus mc1R gene for melanocortin 1 receptor, breed Camosciata delle Alpi
Length=1276
Score = 1221 bits (661), Expect = 0.0
Identities = 666/668 (**99%**), Gaps = 1/668 (0%)
Strand=Plus/Plus

```
Query  2    ACCCCCAGCCACCCTCCCCCTCACACTGGCCCCCAATCGGACAGGGCCCCAGCTGCCTGG  61
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  89   ACCCCCAGCCACCCTCCCCCTCACACTGGCCCCCAATCGGACAGGGCCCCAG-TGCCTGG  147

Query  62   AGGTGTCCATCCCTGACGGGCTCTTTCTCAGCCTGGGGCTGGTGAGTCTTGTGGAGAACG  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  148  AGGTGTCCATCCCTGACGGGCTCTTTCTCAGCCTGGGGCTGGTGAGTCTTGTGGAGAACG  207

Query  122  TGCTGGTGGTGGCTGCCATCGCCAAGAACCGCAACCTGCACTCCCCCATGTACTACTTCA  181
            |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  208  TGCTGGTGGTGGCCGCCATCGCCAAGAACCGCAACCTGCACTCCCCCATGTACTACTTCA  267

Query  182  TCTGCTGCCTGGCCATGTCCGACCTGCTGGTGAGCGTCAGCAACGTGCTGGAGACGGCAG  241
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  268  TCTGCTGCCTGGCCATGTCCGACCTGCTGGTGAGCGTCAGCAACGTGCTGGAGACGGCAG  327

Query  242  TCATGCTGCTGCTGGAGGCTGGTGTCCTGGCCACCCGGGCGGCCGTGGTACAGCAGCTGG  301
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  TCATGCTGCTGCTGGAGGCTGGTGTCCTGGCCACCCGGGCGGCCGTGGTACAGCAGCTGG  387

Query  302  ACAATGTCATTGACGTGCTCATCTGCAGCTCCATGGTGTCCAGCCTCTGCTTCCTGGGTG  361
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  ACAATGTCATTGACGTGCTCATCTGCAGCTCCATGGTGTCCAGCCTCTGCTTCCTGGGTG  447

Query  362  CCATCGCTGTGGACCGCTACATCTCCATCTTCTACGCCCTGCGGTACCACAGTGTCGTGA  421
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  CCATCGCTGTGGACCGCTACATCTCCATCTTCTACGCCCTGCGGTACCACAGTGTCGTGA  507

Query  422  CACTGCCCCGCGCGTGGAGGATCATTGCAGCCATCTGGGTGGCCAGCATCCTCACCAGCG  481
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  CACTGCCCCGCGCGTGGAGGATCATTGCAGCCATCTGGGTGGCCAGCATCCTCACCAGCG  567

Query  482  TGCTCTCCATCACCTACTACAACCACACGGTCGTCCTGCTGTGCCTCGTTGGCTTCTTCA  541
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  TGCTCTCCATCACCTACTACAACCACACGGTCGTCCTGCTGTGCCTCGTTGGCTTCTTCA  627

Query  542  TAGCCATGCTGGCCCTGATGGCCGTCCTCTATGTCCACATGCTGGCCCGGGCCTGCCAGC  601
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  628  TAGCCATGCTGGCCCTGATGGCCGTCCTCTATGTCCACATGCTGGCCCGGGCCTGCCAGC  687

Query  602  ATGCCCGGGGCATCGCCCGGCTCCAGAAGAGGCAGCGCCCCATTCATCAGGGCTTTGGCC  661
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  688  ATGCCCGGGGCATCGCCCGGCTCCAGAAGAGGCAGCGCCCCATTCATCAGGGCTTTGGCC  747

Query  662  TCAAGGGC   669
            ||||||||
Sbjct  748  TCAAGGGC   755
```

Appendix 6: Basic Local Alignment Search Tool (BLAST) output of the *MC1R* amino acid sequence

AltName: Full=Melanocortin receptor 1; Short=MC1-R
emb|CAA74292.1| melanocyte stimulating hormone receptor [Ovibos moschatus] emb|CAR82366.1| melanocortin 1 receptor [Capra hircus]
Length=317

Sort alignments for this subject sequence by:
E value Score Percent identity
Query start position Subject start position

Score = 440 bits (1132), Expect = 1e-128, Method: Compositional matrix adjust.
Identities = 221/222 (99%), Positives = 222/222 (**100%**), Gaps = 0/222 (0%)

```
Query  8    PPATLPLTLAPNRTGPQCLEVSIPDGLFLSLGLVSLVENVLVVAAIAKNRNLHSPMYYFI  67
            PPATLPLTLAPNRTGPQCLEVSIPDGLFLSLGLVSLVENVLVVAAIAKNRNLHSPMYYFI
Sbjct  18   PPATLPLTLAPNRTGPQCLEVSIPDGLFLSLGLVSLVENVLVVAAIAKNRNLHSPMYYFI  77

Query  68   CCLAMSDLLVSVSNVLETAVMLLLEAGVLATRAAVVQQLDNVIDVLICSSMVSSLCFLGA  127
            CCLAMSDLLVSVSNVLETAVMLLLEAGVLATRAAVVQQLDNVIDVLICSSMVSSLCFLGA
Sbjct  78   CCLAMSDLLVSVSNVLETAVMLLLEAGVLATRAAVVQQLDNVIDVLICSSMVSSLCFLGA  137

Query  128  IAVDRYISIFYALRYHSVVTLPRAWRIIAAIWVASILTSVLSITYYNHTVVLLCLVGFFI  187
            IAVDRYISIFYALRYHSVVTLPRAWRIIAAIWVASILTSVLSITYYNHTVVLLCLVGFFI
Sbjct  138  IAVDRYISIFYALRYHSVVTLPRAWRIIAAIWVASILTSVLSITYYNHTVVLLCLVGFFI  197

Query  188  AMLALMAVLYVHMLARACQHARGIARLQKRQRPIHQGFGLQG  229
            AMLALMAVLYVHMLARACQHARGIARLQKRQRPIHQGFGL+G
Sbjct  198  AMLALMAVLYVHMLARACQHARGIARLQKRQRPIHQGFGLKG  239
```

Appendix 7: Basic Local Alignment Search Tool (BLAST) output of the *ASIP* nucleotide sequence

>emb|AM746057.2| Capra hircus partial asip gene for agouti signaling protein, exons 2-4
Length=1105

Score = 521 bits (282), Expect = 3e-152
Identities = 289/292 (**99%**), Gaps = 2/292 (1%)
Strand=Plus/Plus

```
Query  1    GCA-ATGAATTTG--AGACCCCTGGCCTACCTGACTGCCTTCTCTGTCGCTCTCAAGCCT  58
            ||| ||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21   GCACATGCATTTGCCAGACCCCTGGCCTACCTGACTGCCTTCTCTGTCGCTCTCAAGCCT  80

Query  59   CCTGGGATGGATGTCAGCCGCCTCCTCCTGGCTACCTTGCTGGTCTGCCTGTGCTTCCTC  118
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  81   CCTGGGATGGATGTCAGCCGCCTCCTCCTGGCTACCTTGCTGGTCTGCCTGTGCTTCCTC  140

Query  119  AGTGCCTACAGCCACCTGGCACCTGAGGAAAAGCCCAGAGATGAAAGGAACCTGAAGAAC  178
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141  AGTGCCTACAGCCACCTGGCACCTGAGGAAAAGCCCAGAGATGAAAGGAACCTGAAGAAC  200

Query  179  AATTCTTCCATGAACCTGTTGGATTTCCCTTCTGTCTCTATTGTGGGTAAGTAGCCTGGC  238
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  201  AATTCTTCCATGAACCTGTTGGATTTCCCTTCTGTCTCTATTGTGGGTAAGTAGCCTGGC  260

Query  239  CTGGGGCCCAGCCTCTGGGCTCTGGCCCATGAGAAGGGGCTGCAGGGGGTCA   290
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261  CTGGGGCCCAGCCTCTGGGCTCTGGCCCATGAGAAGGGGCTGCAGGGGGTCA   312
```

Appendix 8: Basic Local Alignment Search Tool (BLAST) output of the *ASIP* amino acid sequence

>emb|CAN88844.2| agouti signaling protein [Capra hircus]
Length=167

Sort alignments for this subject sequence by:
E value Score Percent identity
Query start position Subject start position

Score = 86.7 bits (213), Expect = 6e-23, Method: Compositional matrix adjust.
Identities = 52/53 (98%), Positives = 52/53 (**98%**), Gaps = 0/53 (0%)

```
Query  1   MYVSRLLLATLLVCLCFLSAYSHLAPEEKPRDERNLKNNSSMNLLDFPSVSIV  53
           M VSRLLLATLLVCLCFLSAYSHLAPEEKPRDERNLKNNSSMNLLDFPSVSIV
Sbjct 1 MDVSRLLLATLLVCLCFLSAYSHLAPEEKPRDERNLKNNSSMNLLDFPSVSIV 53
```

Appendix 9: Estimated Marginal Means of Body Traits Based on Genotypes at *g.201G>A*

Dependent Variable	GENO TYPE	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
BODYWEIGHT	1.00	20.925	.824	19.289	22.561
	2.00	12.750[a]	4.416	3.985	21.515
BODYLENT	1.00	76.694	1.133	74.446	78.942
	2.00	64.067[a]	6.068	52.023	76.111
TAILLENT	1.00	11.606	.193	11.223	11.989
	2.00	11.200[a]	1.033	9.149	13.251
HEIGHTWITHERS	1.00	48.697	1.307	46.102	51.291
	2.00	32.500[a]	7.005	18.598	46.402
HEIGHTRUMP	1.00	50.835	1.314	48.228	53.442
	2.00	50.167[a]	7.039	36.197	64.136
HEARTGIRTH	1.00	61.756	.854	60.062	63.451
	2.00	57.333[a]	4.575	48.253	66.414
CHESTDEPTH	1.00	24.116	.409	23.304	24.928
	2.00	22.600[a]	2.193	18.248	26.952
HIPWIDTH	1.00	10.504	.194	10.119	10.890
	2.00	9.400[a]	1.041	7.335	11.465
FORCANLENT	1.00	12.026	.192	11.645	12.407
	2.00	12.233[a]	1.028	10.194	14.273
EARLENT	1.00	10.592	.210	10.174	11.009
	2.00	12.417[a]	1.126	10.181	14.652

a. Based on modified population marginal mean. GG is coded as 1 while AG is coded as 2.

Appendix 10: Estimated Marginal Means of Morphological Traits Based on Genotypes at *g.206C>A*

Dependent Variable	genotype	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
bodyweight	1.00	20.741	.828	19.099	22.384
	2.00	20.000[a]	7.742	4.636	35.364
bodylength	1.00	76.412	1.136	74.158	78.666
	2.00	80.000[a]	10.625	58.915	101.085
taillength	1.00	11.583	.191	11.203	11.963
	2.00	11.000[a]	1.791	7.446	14.554
withersheight	1.00	48.487	1.316	45.876	51.099
	2.00	48.000[a]	12.307	23.577	72.423
rumpheight	1.00	50.760	1.299	48.182	53.337
	2.00	50.000[a]	12.148	25.892	74.108
heartgirth	1.00	61.591	.850	59.904	63.278
	2.00	61.500[a]	7.951	45.721	77.279
chestdepth	1.00	24.056	.405	23.253	24.860
	2.00	24.500[a]	3.787	16.984	32.016
hipwidth	1.00	10.451	.193	10.067	10.835
	2.00	12.000[a]	1.809	8.410	15.590
forcanbonelent	1.00	12.024	.191	11.645	12.402
	2.00	11.000[a]	1.783	7.461	14.539
earlength	1.00	10.586	.212	10.165	11.006
	2.00	12.000[a]	1.982	8.067	15.933

a. Based on modified population marginal mean.

CC is coded as 1 while CA is coded as 2.

Appendix 11: ESTIMATED MARGINAL MEANS BASED ON GENOTYPES AT *g.221T>C*

Dependent Variable	GENOTYPE	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
bodyweight	1.00	20.607	1.071	18.480	22.735
	2.00	23.623	1.913	19.822	27.424
	3.00	17.750[a]	4.053	9.697	25.803
bodylength	1.00	75.915	1.469	72.997	78.833
	2.00	78.544	2.624	73.331	83.756
	3.00	76.500[a]	5.559	65.456	87.544
tailength	1.00	11.680	.248	11.189	12.172
	2.00	11.940	.442	11.062	12.819
	3.00	10.667[a]	.937	8.805	12.528
withersheight	1.00	47.619	1.726	44.190	51.048
	2.00	53.786	3.083	47.660	59.911
	3.00	49.750[a]	6.532	36.772	62.728
rumpheight	1.00	50.223	1.688	46.870	53.576
	2.00	55.681	3.015	49.691	61.670
	3.00	53.000[a]	6.387	40.311	65.689
heargirth	1.00	61.603	1.114	59.389	63.817
	2.00	63.033	1.991	59.078	66.988
	3.00	61.333[a]	4.218	52.954	69.712
chestdepth	1.00	23.977	.532	22.920	25.035
	2.00	24.465	.951	22.576	26.353
	3.00	24.167[a]	2.014	20.165	28.168
hipwidth	1.00	10.322	.254	9.817	10.826
	2.00	10.591	.453	9.690	11.492
	3.00	10.250[a]	.961	8.341	12.159
forcanonlength	1.00	11.870	.250	11.374	12.366
	2.00	12.339	.446	11.453	13.225

	3.00	11.500[a]	.945	9.623	13.377
earlength	1.00	10.280	.263	9.757	10.804
	2.00	10.944	.471	10.009	11.878
	3.00	12.500[a]	.997	10.520	14.480

a. Based on modified population marginal mean.

TT is coded as 1, TC=2 and CC=3

Appendix 12: List of Amino Acids and Their Abbreviations

Nonpolar Amino Acids (Hydrophobic)

Amino acid	**Three letter code**	**Single letter code**
Glycine	Gly	G
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Phenylalanine	Phe	F
Tryptophan	Trp	W
Proline	Pro	P

Polar (Hydrophilic)

Serine	Ser	S
Threonine	Thr	T

Cysteine	Cys	C
Tyrosine	Tyr	Y
Asparagine	Asn	N
Glutamine	Gln	Q

Electrically Charged (negative)

Aspartic acid	Asp	D
Glutamic acid	Glu	E

Electrically Charged (positive)

Lysine	Lys	K
Arginine	Arg	R
Histidine	His	H

Appendix 13: Nucleotides and Their Abbreviations

IUPAC nucleotide code	**Base**
A	Adenine
C	Cytosine
G	Guanine
T (or U)	Thymine (or Uracil)