1. Introduction

The goat (*Capra Hircus*) is thought to have been first domesticated in Iran in the Zagros Mountains around 8,000BC (Zeder et al., 2000). This region is responsible for the domestication of several common agricultural crops and animals which include wheat, barley, sheep and pigs (Zeder et al., 2000). The first known civilisations (Mesopotamia) also occurred in this region thus making the goat a central figure in all parts of their life, religion, agriculture, nutrition etc. (Boyazoglu et al., 2005). Animal domestication is thought to have occurred in at least twelve geographical areas around the world. Archaeology and genetics have been used together in documenting the locations of livestock domestication (Zeder et al., 2006). Domestic goats (*Capra hircus*) are closely related to the Bezoar (*Capra aegagrus*)and show a low degree of phylogeographic structure when compared with other artiodactykla species such as cattle (*Bos taurus*) and pig (*Sus scrofa*) (Manceau et al., 1999 and Luikart et al., 2001). Weak phylogeographic structure among goat populations has been attributed to long term gene flow between distant geographical regions, which might have been favoured by transportation of domestic goats as a source of food in human migration, trade routes and exploratory journeys (Luikart et al., 2001).

Neolithic farmers are credited with introducing the goat into Ireland. The Neolithic age in Ireland is thought to have occurred in Ireland around 4,000 to 2,500 BC. Farming was the main element of this age that was introduced into Ireland. They brought with them cows, goats and sheep. (www.wesleyjohnston.com/users/ireland/past/pre_norman_history/neolithicage). The map on Figure 1 shows the path that the goat took through Europe which shows the goat being brought upwards from France to Ireland originally.

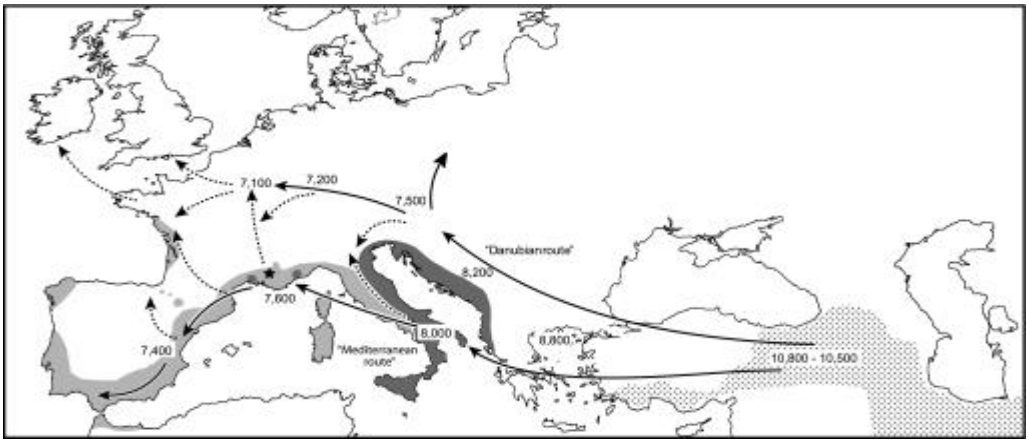


Figure 1- Map shows occidental part of the current geographic distribution of the wild goat, *Capra aegagrus* (dotted area), as well as the two main waves for the initial advancement of the Neolithic culture into Europe: the Mediterranean route and the Danubian route. The location of Baume d'Oullen is indicated by a star. The dates on the map are calibrated radiocarbon date-derived B.P. (cal. B.P.). Solid-line arrows indicate main flow; broken-line arrows indicate possible secondary flows. Dark gray zones indicate the area of the Impressa culture (8,000–7,500 cal. B.P.); light gray zones indicate the area of the Cardial and cultures (between 7,500 and 6,800 cal. B.P.) Guilaine J et al., 2003)

The Bilberry Goat have gained their name through the area they inhabit in Waterford city known as the Bilberry Rock. Locals believe that French Huguenots in the late seventeenth century imported the goats into Waterford city when they were fleeing from France. According to local historians the Bilberry goat has inhabited the area for over a hundred years. The Bilberry goat herd was first recorded in 1876 in a walled area in Waterford city known as the Bilberry rock. This area the bilberry rock is distinguished by its soil type which is made up of several layers of quartz and iron ore. (Ryland, R. H et al., 2011). In the past number of years this area has been reduced by up to 50% due to expansion of the city into the area.

The first recording of the herd showed that there were five males and two females which were in 1990. The population has now increased to 84 according to the latest census in 2007 with the herd consisting of 35 male goats and 49 female goats. The pre-dominant characteristics of the

breed are the body is all cream/coffee cream in colour, the goats have a chocolate /brown tail and they have a brown face. The goats in appearance have shaggy hair and have sturdy bodies. Their horns are big and heavy and are shaped differently to others in Europe. Legs are straight and strong. Due to the area they inhabit it is thought that the herd has had little introgression from other breeds in the last few years.

The Old Irish Goat is described as a small animal with a deep body that is set on short legs. Its coat is medium in length to long and shaggy; the ears small and pricked; the head long and dished. The four basic colours of goats can all be represented tan, black, grey and brown. The tan colour can vary from white looking through to yellow, fawn, golden and red to chestnut. Colour patterns are very common with light belly, dark belly, no pattern tan and no pattern black or brown (three shades). Lateral stripes have also been observed. White patterning is not a feature in the coat colour of the Old Irish Goat breed. The first settlers to the Burren came in the Neolithic period in the form of farmers who brought both agriculture and animal husbandry to the Burren and also trading stations. The neoliths brought with them goats as previously discussed as they were hardy, multipurpose and low- maintenance animals.

Small local farmers generally kept domestic goat stock in the Burren in small herds of 10-20 that would roam around the mountains the higher parts of the farm that would be less productive and dogs would bring them down for milking if required. These domestic herds would have been allowed to mingle with the wild goats. Hence, introgression between the herds was happening throughout the 1990s. The reduction in numbers around the Second World War was thought to have been the point where domesticated breeds had to be introduced into the area. In the current herds observed in the Burren a selection of domesticated breeds of goats and Old Irish type of goat can be seen throughout the area. As the truly feral groups have frequented the Burren for some time but the traditional method of goat keeping has meant that these goat herds have been constantly replenished from domestic stock.(Porter V, 1996)

The other goat breeds chosen for this study were the Saanen, Toggenburg, British Alpine and the Anglo Nubian Goats. These goat breeds are among the top eight goat breeds in the world according to figures released from the Food and Agricultural Organisation of the European Union.

The Anglo Nubian goat is a goat breed that was originally developed in England by crossing goats bred in England and goats that were imported from the east. The goats came from the east at the end of the 19th century on steam ships providing fresh milk to the passengers and were bought upon docking in England. These foreign goats were known as Nubians. At the turn of the 20th century four male Nubians were brought to England and it is descendants from these four males that led to the set up of the Anglo Nubian section of the British Goat Society herd book in 1910. The Anglo Nubian goat is described by the Anglo Nubian society as' an alert sound well balanced animal standing four square and possessing the capacity and breeding ability of a good milking goat with a high proud head carriage and majestic bearing'. The Anglo-Nubian goat has long, pendulous ears and the nose is convex in shape. The body shape has a pronounced brisket and high withers. The coat can be chestnut, fawn, black, white or cream and it is short and glossy.

The Toggenburg goat herd book in Britain formed in 1925. Toggenburg is a region in Switzerland where this breed of goat is known to have originated from. According to the British Toggenburg society the initial breeding of the British Toggenburg took place between 1884 and 1897 when pure imported Toggenburg males were crossed with indigenous goats. The British breed of Toggenburg were larger in size, greater milk yields and shorter coats and a wider range in coat colour while keeping their original swiss markings. The Toggenburg goat has a compact body and a well attached udder. Its face should be straight of dished a roman nose should not feature in this breed. Colour varies from light fawn to dark chocolate. They also have white markings specifically located in these locations white ears, two white stripes on face, white half ways up their legs and a white triangle around their tail.

The British Alpine goat is known for its quality of milk. Although no date is available for when the alpine goat was introduced into the Ireland, the breed was introduced into Britain in the early 1900's. The original female goat was imported to England from Paris zoo in 1903. The phenotypic breed standards that are set out by the British Alpine society are as follows; the head should be refined with eyes that are full bright and alert. The neck should blend neatly into the shoulders and should be long and slender. The legs should have strong pasterns and should have sound feet. The chest should be full and deep. Front and rear legs should have good space in between each pair. The back should be straight from shoulders to hips with a slight slope from hips to tail. The coat should be made up of fine, short and soft hair.

Saanen goats are named after the Saanen valley in the south Canton Berne in Switzerland. In 1893 several thousand head were taken and spread around Europe. While no date of origin is available for Ireland the first Saanen to be imported into Britain came from Holland in 1922. Saanen goats are known for the volume of milk produced, producing on average a gallon of milk a day. The British goat Society breed standards for the Saanen breed are as follows the body should have good length and depth. They are medium to large in size (weighing approximately 145lbs/65kg). The coat should be short and fine with a supple skin and a fringe over the spine and thighs. The coat colour should be white or light cream in colour but white is preferred.

Genetic diversity is the raw material upon which natural selection acts on to bring about adaptation and evolution to cope with environmental change. Loss of genetic diversity reduces evolutionary potential and is also associated with reduced reproductive fitness. Environmental change is a continuous process and genetic diversity is required for populations to evolve to adapt to such change. Loss of genetic diversity is usually associated with inbreeding and overall reduction in reproduction and survival fitness (Frankham et al., 2004). Maintenance of genetic diversity in livestock species depends upon the definition and implementation of appropriate conservation and sustainable management programs, which should be based on comprehensive information regarding the structure of the populations, including sources of genetic variability

among and within-breeds (Notter,1999) Genetic markers such as microsatellites provide an extremely good source of information to genetically characterise breeds and to analyse population structure and relationships (Oliveira et al., 2000). Microsatellites markers have been used for evaluating genetic variability and estimating genetic distances among closely related populations in numerous studies of ruminant species (Moore et al., 1991; Buchanan et al., 1994; Kemp et al., 1995).

Autosomal microsatellites have been isolated in large numbers from most livestock species. FAO/ISAG (International Society of Animal Genetics) publishes recommended lists of autosomal microsatellite markers for genetic diversity studies for each species. (http://www.fao.org/dad-is). Autosomal microsatellite loci are commonly used for genetic identification of individuals in a population, population diversity estimations, and differentiation of populations, calculations of genetic distances, estimation of genetic relationships, and the estimation of population genetic admixture.

Microsatellites otherwise known as variable number of tandem repeats (VNTRs) or short tandem repeat polymorphisms (STRs) are repeats of a small motif usually one to six bases with the most common repeat being a CA or a GT (Hughes et al.,2007). The polymorphic content of a microsatellite increases with the increasing length of the repeat. The small size of microsatellites makes them easily amplified by Polymerase Chain Reaction (PCR) and they also have a high rate of polymorphism which makes them a very informative tool for genotyping (Hughes et al., 2007).

MtDna contains highly informative polymorphic sites and its simple maternal inheritance without recombination which makes mtDNA useful for population studies in many organisms (Bradley et al., 1996). The uniparental mode of inheritance, high mutation rate and lack of recombination turns mtDNA into an excellent tool for the study of populations that may have complex genetic histories as in several domestic species cattle (Bradley et al., 1996) dog

(Savolainen et al., 2002) horse (Jansen et al., 2002), pig (Guiffra et al., 2000) and sheep (Hiendleder et al., 2002). Mitochondrial DNA in animals has a rate of evolution that is five to ten times greater than in the nuclear genome, making mtDNA ideal for studying the divergence between wild and domestic populations under the relatively short timescale of domestication (i.e. <10,000 years) (Zeder et al., 2006).

Analysis of genetic variation at the mitochondrial loci has played a key role in investigating the phylogenetic relationships among different Caprine species and populations. Molecular techniques have proved useful in the investigation of the origin and domestication of livestock species and their subsequent migrations as well as providing information on evolutionary relationships (phylogenetic trees) and identifying geographical areas of admixture among populations of different genetic origins. There are two assumptions when using genetic markers

(1) That the polymorphisms observed at the molecular markers are neutral

(2) The use of a relatively small number of independently segregating marker loci is a good predictor of the overall genomic diversity of a population.

The variations in allele frequencies between populations will reflect the distribution of genetic diversity within and amongst populations (Hanotte et al., 2005)

2. Materials and methods

2.1.1 Genetic Diversity

Genetic diversity is the variety of alleles and genotypes present in the group under study (population, species or group of species) (Frankham et al., 2004). This variety of alleles and genotypes can be observed in a number of ways – DNA mutations, recombination's and random as well as directed changes in allele frequencies are observed to reconstruct evolutionary, ecological, historic or demographic processes (divergence, expansions, bottlenecks, genetic

isolation, introgression, adaptation and selection: Hey &Machado, 2003). Domestication of a wild species only partially captures some of the variation of the wild species and the creation of a breed creates genetic subdivision at different levels with variable degrees of gene flow. High genetic diversity allows a population to be able to cope with environmental changes and low genetic diversity can indicate high levels of inbreeding reducing the population's ability to survive changes in its environment.

Molecular techniques allow for the measurement of genetic diversity. Diversity within the breeds is measured with the mean observed and expected heterozygosity averaged over typed loci, the average number of alleles and the allelic richness per locus. Heterozygosity is the probability that a given individual randomly selected from a population will be heterozygous at a given locus. Expected heterozygosity (H_e) measures the probability that two alleles drawn at random from the population are different (Nei et al., 2000). Observed Heterozygosity (H_o) is the proportion of sampled individuals that are heterozygotes.

Allelic richness is the number of alleles corrected for the sample size which is more sensitive to reductions of the effective population size (Ne) than heterozygosity (Luikart & Cornuet, 1998). Polymorphic information content (PIC) of choosen microsatellites allows for the measurement of usefulness of the particular molecular marker. Allele frequencies measure each locus for each sample for the individuals that are typed.

Inbreeding is the most relevant consideration for genetic management. Mating among relatives (inbreeding) is a very common problem in small closed populations (Frankham et al., 2004). Inbreeding results in reductions in heterozygosity, reduced reproduction and survival and to increased risk of extinction (Frankham et al., 2004). Loss of reproductive fitness in a population due to inbreeding is known as inbreeding depression. Inbreeding depression, or the reduction of fitness of populations by inbreeding, is caused mainly by homozygosity of deleterious mutations (Charlesworth & Willis, 2009).

Inbreeding is measured by the inbreeding coefficient of (F_{IS}). As F_{IS} is a probability it ranges from -1 in outbred individuals to 1 in completely inbred individuals. It is related to Wrights F-statistics used for population sub-structure. Wright F_{IS} inbreeding coefficient is defined as the proportion of the total inbreeding within a population that is because of inbreeding within subpopulations (Frankham et al., 2002). F_{IS} is usually estimated from the heterozygote deficit 1 minus the ratio of observed and expected heterozygosity. A positive $F_{is\ indicates}$ possible inbreeding and may also be the result of genetic subdivision (the Wahlund effect) and negative estimates can arise from crossbreeding.

Population expansions and bottlenecks are common during the history of livestock breeding. Population bottlenecks result in loss of alleles, reduced genetic diversity and random changes in allele frequencies. Multiple subdivisions of populations are created in populations by geographical isolation and selective breeding. The relationships among breeds is a major focus of most of molecular studies (Groeneveld et al., 2010)

Sub-population components are measured using Wrights F_{st}. There are a number of ways to calculate F_{st}. The most common method is Cockerham & Weir (1984) takes into account multiple loci and unequal sample sizes across sub populations. Fst values between 0.05 and 0.3 are typical for differentiation of livestock breeds, with a value over 0.15 indicating significant differentiation (Frankham et al., 2002)

2.1.2 Division of breeds

Cluster algorithms identify groups of related individuals without reference to prior information of the genetic subdivisions into for example breeds (Lenstra et al., 2012). The widely used structure program (Pritchard et al., 2000) is used to calculate these cluster algorithms. The Markov-Chain Monte Carlo algorithm, multi-locus genotypes are divided into a user defined number of clusters (k) by assuming base populations in HWE and markers in linkage

equilibrium. The individual genome that belongs to each cluster are estimated by a Bayesian procedure, which allows for the choice between different ancestry models – admixture, no admixture, linkage, spatial correlation, prior population information and between independent and correlated allele frequencies (Lenstra et al.,2012).

2.1.3 Hardy –Weinberg Equilibrium

The measurement of Hardy-Weinberg equilibrium is highly important to conservation genetics as it detects deviations from random mating, testing for selection, modelling the effects of inbreeding and selection, and estimating the allele frequencies at loci showing dominance. The equilibrium is achieved where there is a large population where mating is random and there is no mutation, migration and selection. Allele and genotype frequencies attain equilibrium after just one generation (Frankham et al., 2007). Hardy-Weinberg equilibrium is expected for all loci. There are certain assumptions associated with Hardy Weinberg equilibrium (Frankham et al., 2007). These include

- A large population size
- A closed population (no migration)
- No mutation
- Normal Mendalian segregation of alleles
- Equal fertility of parent genotypes
- Random union of gametes
- Equal survival of all genotypes

2.2 Samples

Hair samples were collected from each goat from the different breeds to be studied. A total of ninety nine individual goats were sampled- Sixteen Anglo Nubian (AN), six British Alpine (BA), eighteen Bilberry (BY), twelve Old Irish Goat (OIG), forty Saanen (SA) and seven Toggenburg (TOG). A clean dry sample of hair containing 20-30 hairs with follicles was taken from each goat. Samples were received at the lab, assigned individual laboratory numbers and stored away from direct sunlight at room temperature. From the individual samples 4-6 hairs with the best follicles were selected, and cut into individual 0.5ml eppendorf tubes and then underwent a Tris-Potassium Chloride (KCL) buffer extraction. This extraction is used to extract the genomic DNA from the hairs which contains both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). A NANODROP 1000 spectrophotometer was used to quantify the amount and quality of the double stranded DNA in each sample post extraction. Each sample was then standardised to a concentration of 20ng/µl using HPLC water.

A total of 10 microsatellites were selected from the published list of markers from the DAD-IS selection and also some from the ISAG list of markers. DAD-IS stands for the Domestic Animal Diversity Information System. It provides information and tools for the management of countries animal genetic resources (AnGr). It provides a list of microsatellites in descending order that countries should use in the characterisation of different populations of animals. ISAG – The International Society of Animal Genetics has a recommended list of microsatellites for each breed to be used for parentage testing of animals. Table 1 - shows the microsatellite information which includes the sequence, source and list for goats.

Table 1- Microsatellite information which includes the forward and reverse sequence, the size range and the dye it was labelled with.

Microsatellite Marker	Forward Sequence 5'-3'	Reverse Sequence 5'-3'	Dye Label	Size range (BP)
ILSTS19	AGGGACCTCATGTAGAAGC	ACTTTTGGACCCTGTAGTGC	NED	148-158
INRA63	GACCACAAAGGGATTTGCACAAGC	AAACCACAGAAATGCTTGGAAG	NED	173-179
SRCRSP8	TGCGGTCTGGTTCTGATTTCA	CCTGCATGAGAAAGTCGATGCTTAG	NED	209-235
CSRD247	GGACTTGCCAGAACTCTGCAA	CACTGTGGTTTGTATTAGTCAGG	VIC	221-247
MAF65	AAAGGCCAGAGTATGATGCAATTAGGAG	CCACTCCTCCTGAGAATATAACATG	VIC	121-157
SPS113	CCTCCACACAGGCTTCTCTGACTT	CCTAACTTGCTTGAGTTATTGCCC	NED	130-160
ILSTS87	AGCAGACATGATGACTCAGC	CTGCCTCTTTTCTTGAGAGC	PET	137-155
SCRSP5	GGACTCTACCAACTGAGCTACAAG	TGAAATGAAGCTAAAGCAATGC	PET	158-180
OARFCB20	GGAAAACCCCCATATATACCTATAC	AAATGTGTTTAAGATTCCATACATGTG	NED	93-109
Inra 5	TTCAGGCATACCCTACACCACATG	AAATATTAGCCAACTGAAAACTGGG	NED	118-126

The ten microsatellites were amplified in two separate PCR multiplexes depending on their fragment sizes which is the number of repeat motifs and the dyes they were labelled with. Multiplex 1 consisted of ILSTS 19, INRA 63, SCRSP 8, CSRD 247, MAF 65 and Multiplex 2 consisted of INRA 5, OARFCB 20, SCRSP 5, SPS113 and ILSTS 87. Forward primers were labelled with florescent dyes all synthesised by Applied Biosystems (http://www3.appliedbiosystems.com/AB_Home/index.htm). These multiplexes were amplified using standard PCR ready-mix methods. This ready-mix method used for each multiplex, StockMarks® Applied Biosystems (ABI) PCR buffer (100mM Tris-HCL, 500mM KCL, 15mM $MgCl_2$ pH 8.3), GeneAmp® ABI 10mM dNTP mix (2.5mM of each nucleotide), AmpliTaq Gold® polymerase (5U/µl), HPLC H_2O and the 5 forward and reverse oligonucleotide primers (20µM) to each multiplex. Outlined in Table 2 are the volumes used of the reagents above for each multiplex per sample.

Table 2-Volumes of reagents for each multiplex

Reagents	**Multiplex 1 and 2 (5 primers)**
StockMarks® Applied Biosystems (ABI) PCR buffer	1.5µl
GeneAmp® ABI 10mM dNTP mix	2µl
AmpliTaq Gold® polymerase	0.25µl
HPLC H_2O	2.75µl
Forward Primer (X5)	0.25µl
Reverse Primer (X5)	0.25µl
Template DNA	1µl
	7µl per sample

Table 3 – PCR conditions for multiplex 1

PCR Step	Temperature (°C)	Time (seconds)
1) AmpliTaq Gold ® Polymerase activation	95	780
2) Denaturation	94	45
3) Primer Annealing	56	45
4) Nucleotide Extension	72	100
5) Final Extension	72	3600
6) Storage Step	4	Forever

Table 4 – PCR Conditions for Multiplex 2

PCR Step	Temperature (ºC)	Time (seconds)
1) AmpliTaq Gold ® Polymerase activation	95	780
2) Denaturation	94	45
3) Primer Annealing	61	45
4) Nucleotide Extension	72	100
5) Final Extension	72	3600
6) Storage Step	4	Forever

The following settings were also applied to the DNA Engine® (PTC-200™) with the thermocycler programs outlined above in Table 2.4 and 2.5.

- Control Mode – Block
- Lid Mode-Tracking at 5ºC above sample temperature
- Ramp Rate: 0.5ºC/sec

Following the PCR process samples were electrophorised using a capillary sequencer the ABI PRISM ® 3130xl Genetic Analyser and then the FASTA files created for each sample were then imported into Genemapper ™v4.0 software (Applied Biosystems, ABI).

A 598 base pair (bp) region of the Caprine mitochondrial displacement loop (D-loop) containing the *Caprine hircus* Hyper variable control region which corresponds to the positions 15,653 to 16250 on the complete mitochondrial sequence of reference (Parma P., et al.,2003) was amplified using standard PCR ready-mix methods. Table 5 contains the primer sequences that were used to amplify this region.

Table 5 –Mitochondrial Primer details

Primer Name	Direction	Sequence 5'-3'	Size (nt)
CAPRINE	Forward	CGTGTATGCAAGTACATTAC	20
CAPRINE	Reverse	CTGATTAGTCATTAGTCCATC	21

Preparation of the PCR was carried using the following steps outlined.

A reaction mix was prepared on ice in a 1.5 µl microcentrifuge tube using the following volumes for the reaction mixture as outlined in Table 6

Table 6 - PCR volumes for mitochondrial DNA

Reagent	**Concentration of Working Stock**	**Volume (µl) for 1 sample**
StockMarks® PCR Buffer	100mM Tris-HCL, 500mM KCL, 15mM MgCL2 pH 8	3
dNTP's	0.3125mM of each nucleotide	4
CAP-F	20µM	0.5
CAP-R	20µM	0.5
AmpliTaq Gold ® polymerase	5U/µl	0.5
dH2O		5.5
Template DNA		1
Total Volume		**15**

Plates were transferred to a DNA Engine ® (PTC-200™) Thermocycler and underwent the following cycling conditions as described in Table 7

Table 7 - Cycling conditions for mitochondrial PCR

PCR Step	**Temperature 0C**	**Time**
1)Polymerase Activation	95	15mins
2)Denaturing	94	45secs
3)Primer Annealing	61	45secs
4)Nucleotide Extension	72	1 min20secs
5) Steps 2-4	29 times before proceeding to step 6	
6)Final Extension	72	1hour
7)Storage	4	Forever

After the amplification process the fragments of the D-loop went through a cleanup process using ExoSAP-IT® (USB) to remove excess oligonucleotides that can affect the process downstream. After this process the PCR fragment was sequenced using a BigDye® Terminator v 1.1 Cycle Sequencing Kit (ABI). The end product then underwent

electrophoresis on the ABI PRISM® 3130xl Genetic Analyzer a 36cm 16 capillary array and POP-7™ (ABI) polymer was used. Dye filter settings G5 and E were used for fragment and sequence analysis instrument protocols respectively. Following electrophoresis analysis of the mtDNA was carried out using Sequencing Analysis™ v5.2 software (ABI). A fasta file was created for each sample post-analysis.

Analysed microsatellite data was imported into the Excel Microsatellite Tool Kit V3.1.1 (Park, 2001) available at http://animalgenomics.ucd.ie/sdepark/ms-toolkit/. All allelic sizing data for the Caprine microsatellites was converted into standardise international sizing which was based on the international society of Animal Genetics (ISAG) sizing recommendations. The Excel Microsatellite Tool Kit V3.1.1 (Park, 2001) was used to convert the data into numerous formats required for other microsatellite analysis software programs.

Micro-Checker V2.2.3 (Van Oosterhout et al, 2004) was used to check for null alleles-alleles that were missed by the PCR process, stuttering- allelic sizing changes that occur during PCR and large allele dropout due to larger fragments not amplifying. A .dat file in 3 digit gene pop format was imported into the Micro-checker and this carried out the analysis with a confidence level of 95%.

Genetic diversity as discussed previously is measured by mean number of alleles, expected heterozygosity, observed heterozygosity and polymorphic information content of each locus. Heterozygosity both observed (Ho) and expected (He) was calculated using Arlequin 3.5.1.3(Excoffier, L., 2009).The polymorphic information content (PIC) was calculated using the Excel Microsatellite Toolkit V3.1.1 (Park, 2001). Allelic Richness was calculated using Fstat software available at *www2.unil.ch/popgen/softwares/fstat.htm.* Arlequin 3.5.1.3(Excoffier, L., 2009) was also used to calculate F-statistics of Wright (1969) Fst calculations was used at a confidence level of 95% and 1000 permutations.

Deviations from Hardy Weinberg were calculated using genepop software v. 4.1 available on *http://kimura.univ-montp2.fr/~rousset/Genepop.htm*. P values were estimated using the Guo and Thompson (1992) Markov-Chain algorithm with 1000 dememorization steps for 100 batches and 1000 iterations per batch.

The inbreeding co-efficient Fis (Wright, 1992) was used to measure the heterozygote deficiencies within populations. FIS was measured using the computer software programme FSTAT v (2.9.3.2).

Population expansions and bottlenecks were measured using computer program *BOTTLENECK* which computes for each population sample and for each locus the distribution of the heterozygosity expected from the observed number of alleles (k), given the sample size (n) under the assumption of mutation-drift equilibrium.

Genetic relationships among the goat populations were analyzed using factorial correspondence analysis (FCA) using Genetix v.4.05. The Principal Component Analysis (PCA) plots a set of related variables within a graphical structure to create a visual component in how the variables relate to each other and was calculated using an excel add on called XLSTAT.

The Bayesian clustering algorithm implemented by STRUCTURE v.2.3.1 software (Pritchard et al., 2000) was used to assess the number of ancestral populations underlying the breeds analyzed and the proportion of mixed ancestry among them. The number of assumed populations (K) was evaluated for K values ranging from 2 to 10, and the likelihood of the observed distribution of genotypes given the assumed number of ancestral populations. For each value of K, ten independent analyses were carried out under an admixture model.

Analysed Mitochondrial data for both forward and reverse sequences were imported into a software program called MEGA v4 (Tamura, Dudley, Nei and Kumar, 2007). Using the

CLUSTAL W option a 598 bp region was constructed and aligned for each sample using both the forward and reverse sequences. Once the sequences were aligned, this file was used in DnaSP v5 (Rozas et al, 2003) software program downloaded from http:// www.ub.edu/dnasp/ to calculate singleton variable sites, parsimony variable sites, transitions, transversions, overall haplotype content and haplotype diversity.

ARLEQUIN v3.0 was used to analyse Fu's *Fs* (Fu, 1997) statistics and Tajima's *D* (Tajima's, 1989) statistics. Both Fu's F*s* and Tajimas's D test whether the population sequences conform to expectations of neutrality. Negative F*s* values indicate population demographic expansions. Negative Tajimas's D values indicate low frequency polymorphisms relative to expectations indicating population size expansions after a bottleneck and/or a purifying selection. Positive Tajima's D values indicate a decrease in population size with low levels of both low and high polymorphisms. ARLEQUIN v3.0 is downloadable from http://cmpg.unibe.ch/software/arelequin3/

Mismatch distribution values were calculated using the DnaSP v5 software program which graphically plotted the distribution of observed and expected pairwise nucleotide site differences for each population. Multimodal plots indicate that populations are at demographic equilibrium while unimodal graphs indicate that populations have passed through recent demographic expansion.

A phylogenetic analysis was conducted in MEGA4 (Tamura et al., 2007). The evolutionary history was inferred using the Neighbor-Joining method (Saitou et al., 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein J, 1985). is taken to represent the evolutionary history of the taxa analyzed Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein J, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All

positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 452 positions in the final dataset.

3. Results

3.1 Microsatellite Data

3.1.1 Genetic Diversity

All 10 loci that were analysed were found to be polymorphic with a total number of 74 alleles detected for the ninety nine goats sampled from the six different breeds. The number of alleles detected at each locus varied between 4 (INRA5) and 10 (MAF65) with an overall mean of 7.4 alleles per locus. There was no evidence of null alleles or no evidence of large allele drop out. The Saanen goats had the highest mean number of alleles (MNA) at 6.7 and the Anglo Nubian goats showed the lowest MNA at 3.4. He values ranged from 0.453 for the Anglo Nubian goats to 0.712 for the Toggenburg goats. Ho values ranged from 0.494 for the Anglo Nubian goats to 0.843 for the Toggenburg goats. Polymorphic information content (PIC) for each microsatellite is also included in Table 8. PIC values ranged from 0.420 for INRA 5 to 0.661 SPS 113.

Table 8 - showing Number of alleles, Expected Heterozygosity (He), Observed Heterozygosity (Ho), Polymorphic information content (PIC) per locus for each population

Population		Anglo Nubian				British Alpine				Saanen				Toggenburg				Bilberry				Old Irish Goat			
Locus	No of Alleles	He	Ho	AR	PIC	He	Ho	AR	PIC	He	Ho	AR	PIC	He	Ho	AR	PIC	He	Ho	AR	PIC	He	Ho	AR	PIC
ILSTS19	6	0.28	0.31	2.24	0.25	0.86	0.83	5	0.76	0.6	0.63	3.61	0.55	0.66	0.71	4.57	0.57	0.3	0.28	2.38	0.27	0.61	0.67	3.44	0.53
INRA63	5	0.31	0.25	1.96	0.26	0.71	0.67	3	0.58	0.65	0.65	3.85	0.6	0.67	0.71	3	0.55	0.32	0.39	1.96	0.26	0.59	0.58	3	0.48
SRCRSP8	9	0.52	0.38	2.84	0.45	0.48	0.67	2	0.35	0.65	0.63	4.74	0.62	0.67	1	3	0.55	0.36	0.33	1.98	0.29	0.55	0.75	2.5	0.43
CSRD247	8	0.56	0.56	2.77	0.46	0.76	1	5	0.64	0.72	0.73	4.08	0.66	0.76	0.86	4.7	0.66	0.65	0.78	3.77	0.59	0.78	0.75	5.06	0.71
INRA5	4	0.44	0.63	2	0.34	0.53	0.83	2	0.37	0.53	0.63	2.76	0.46	0.53	0.57	2	0.37	0.6	0.61	2.92	0.52	0.58	0.5	2.76	0.47
MAF65	10	0.49	0.5	2.75	0.42	0.77	0.83	4	0.65	0.82	0.95	5.77	0.78	0.85	1	4.98	0.75	0.53	0.61	2.81	0.45	0.69	0.67	4.98	0.63
OARFCB20	5	0.52	0.63	2	0.38	0.65	0.67	4	0.55	0.73	0.7	3.8	0.67	0.54	0.71	2.86	0.43	0.56	0.5	3.04	0.48	0.66	0.58	3.39	0.56
SPS113	9	0.7	0.75	3.73	0.62	0.76	0.83	6	0.66	0.74	0.68	5.3	0.71	0.82	1	4.85	0.73	0.68	0.78	3.31	0.59	0.72	0.5	4.45	0.65
ILSTS87	9	0.12	0.13	1.62	0.11	0.68	0.83	3	0.55	0.77	0.83	4.82	0.72	0.8	0.86	4.84	0.7	0.45	0.5	2.85	0.39	0.78	0.75	5.15	0.71
SCRSP5	9	0.59	0.81	2.99	0.49	0.74	0.67	4	0.62	0.75	0.68	4.19	0.7	0.82	1	5.57	0.73	0.66	0.72	3.49	0.58	0.63	0.67	4	0.56
Mean		0.453	0.495	2.49		0.694	0.783	3.8		0.696	0.711	4.292		0.712	0.842	4.037		0.511	0.55	2.851		0.659	0.642	3.873	

3.1.2 Hardy Weinberg Equilibrium

All populations were shown to be in Hardy Weinberg equilibrium using the Markov Chain Method to calculate the P-values. No populations showed any deviations from Hardy Weinberg with P-values of <0.05. The p values per locus for each population are outlined in Table 9.

Table 9 – Hardy Weinberg p values per locus for each population. AN (Anglo Nubian), British Alpine (BA), Bilberry (BY), Old Irish Goat (OIG) and Toggenburg (TOGG)

Locus	AN	BA	BY	OIG	SA	TOGG
ILSTS19	1	0.441	0.389	0.892	0.227	0.838
INRA63	0.433	1	1	0.359	0.82	0.437
SRCRSP8	0.129	1	1	0.239	0.163	0.133
CSRD247	0.663	0.525	0.447	0.466	0.074	0.766
INRA5	0.233	0.395	0.689	0.793	0.591	1
MAF65	1	0.195	0.717	0.495	0.285	0.667
Oarfcb20	0.618	1	0.876	0.368	0.127	1
Sps113	0.113	1	0.424	0.399	0.658	0.824
ILSTS87	1	1	0.599	0.401	0.115	0.912
Scrsp5	0.11	0.12	0.132	1	0.105	1

3.1.3 Inbreeding and Outbreeding measures

Inbreeding and outbreeding was measured using the coefficient (Fis) using the FSTAT software. Values for Fis can range from -1 to 1. Excessive heterozygotes are shown in a negative Fis values and are evidence of populations that have outbred. Populations which are deficient in heterozygotes are represented by positive Fis values and are a sign that inbreeding has taken place within the population. The population that showed an overall

positive value was the Old Irish Goats with a Fis value of 0.029. All other populations showed signs of outbreeding with overall negative values for all populations. The lowest Fis value was shown in the Toggenburg goats at -0.202.

Table 10- The Fis values per Population

Fis Per population :	AN	BA	BY	OIG	SA	TOGG
ILSTS1	-0.119	0.038	0.071	-0.093	-0.042	-0.091
INRA63	0.211	0.07	-0.214	0.006	0	-0.071
SRCRSP	0.289	-0.429	0.064	-0.375	0.035	-0.556
CSRD24	-0.007	-0.364	-0.196	0.039	-0.008	-0.143
INRA5	-0.429	-0.667	-0.014	0.148	-0.193	-0.091
MAF65	-0.03	-0.087	-0.154	0.033	-0.167	-0.2
Oarfcb	-0.22	-0.026	0.105	0.125	0.036	-0.364
Sps113	-0.071	-0.111	-0.153	0.32	0.088	-0.235
ILSTS8	-0.034	-0.25	-0.117	0.039	-0.076	-0.075
Scrsp5	-0.393	0.111	-0.097	-0.067	0.101	-0.235
All	-0.092	-0.141	-0.079	0.029	-0.02	-0.202

3.1.4 Genetic Variation within the Breeds

Fst (Wright, 1965) was used to estimate the genetic variation within the populations. Table 11 shows the values for Fst between each population. Fst values ranged from 0.0161 between the British Alpine Goats and the Saanen goats to 0.3638 between the Anglo Nubian goats and the Bilberry goats. Standard Bonferroni corrections were applied to the Fst calculations and all values were significantly different to zero ($P<0.05$) with the exception of the pairings between the British Alpine goats and the Saanen goats, the British Alpine and the Toggenburg goats and the Saanen goats and the Toggenburg goats. Figure 2 shows the PCA- principle component analysis plot for the populations which shows the populations graphed in relation to each other. The plot clearly shows that the Anglo Nubian goats and the Bilberry goats are separate from the other populations.

Figure 3- shows a Factorial Correspondence analysis chart which plots each individual according to their alleles at different loci. This chart shows the Anglo Nubian goats cluster together with the next population being the Bilberry to show individuality from the different populations

Table 11 – Fst values for pairwise differences

	AN	BA	BY	OIG	SA	TOGG
AN	0					
BA	0.26	0				
BY	0.3638	0.1615	0			
OIG	0.2743	0.0863	0.1675	0		
SA	0.2414	0.0161	0.0968	0.0609	0	
TOGG	0.2641	0.036	0.1566	0.0661	0.0232	0

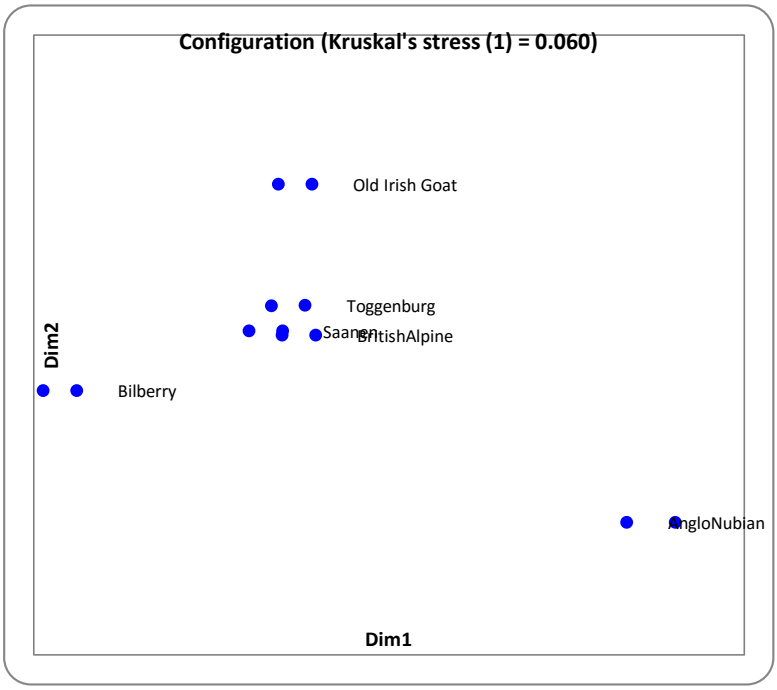


Figure 2 – PCA plot for the populations

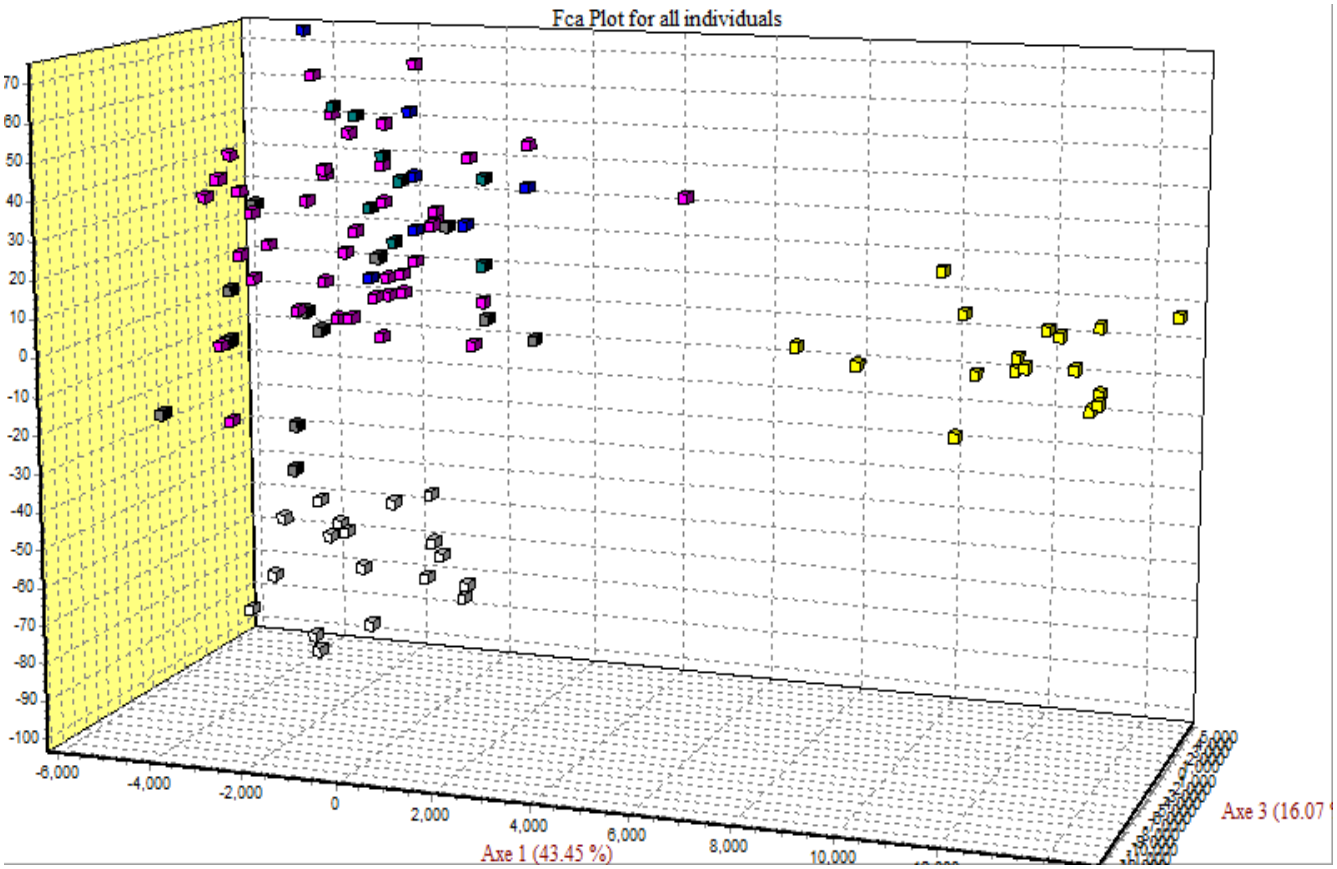


Figure 3- shows a 3d graphical representation of the factorial correspondence analysis using GENETIX (Belkhir et al., 1996) which illustrates the genetic differentiation between the populations in this study. Yellow =Anglo Nubian, White=Bilberry, Pink=Saanen, Blue=British Alpine, Grey=Old Irish Goat, Green=Toggenburg

3.1.5 Population Structure

Population structure was analysed using Structure v2.3.1.The most likely number of clusters/populations (k) was estimated from the maximised modal log likelihood data with standard deviation included. Figure 4 displays plots for the maximised modal log-likelihood with standard deviation bars included. Optimum K values for both estimation methods were achieved at K=3 and K=4. Individuals within the 3 /4 clusters were assigned a percentage

value (Q-Value) for each cluster and these percentages are shown in the form of a bar plot in Figure 5 and 6.

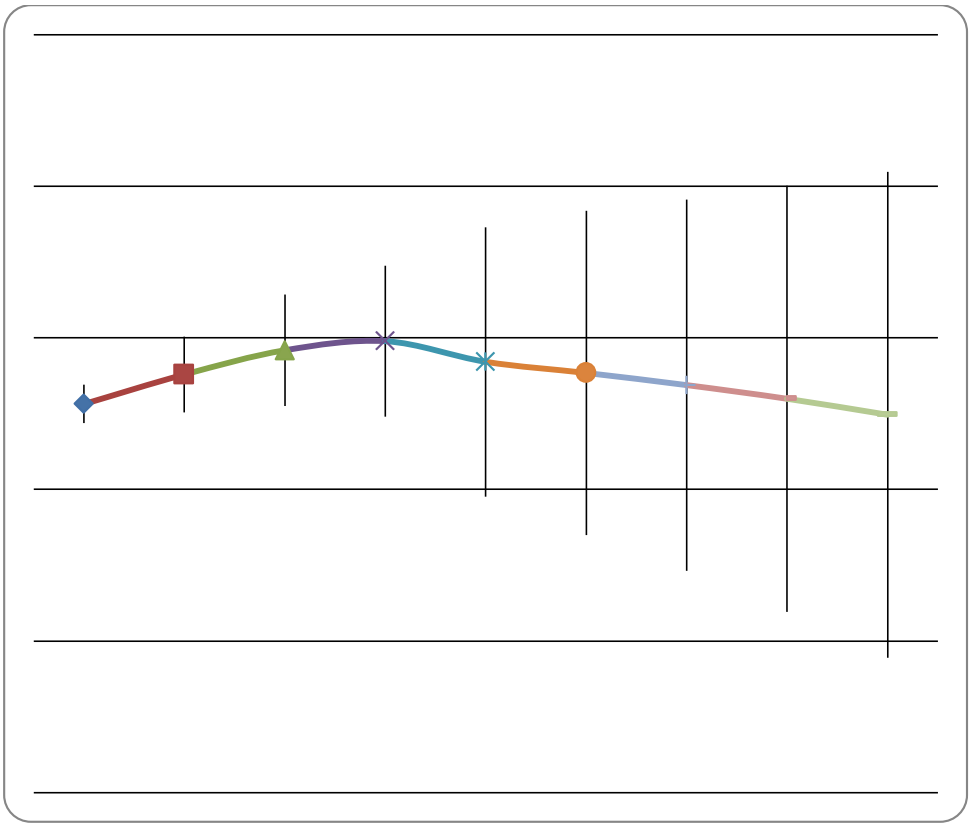


Figure 4 - K estimation plot based on LnP(D) averages for each run along with overall standard deviation (x10).

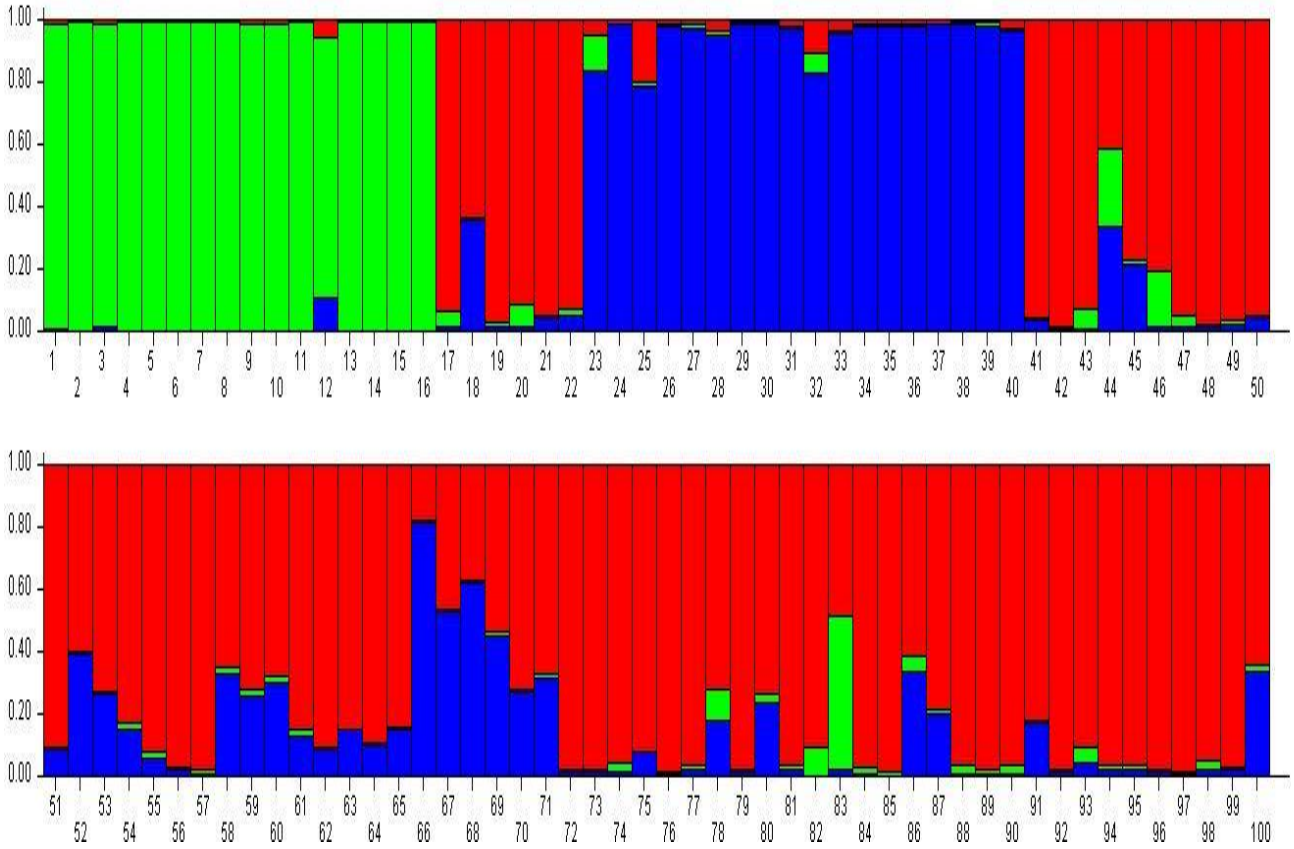


Figure 5 - Bar plot showing the population assignment percentage (Q-Values) of each individual within K=3 clusters*.

**** Sample Identifications are as follows,Anglo Nubian 1-16, British Alpine 17-22, Bilberry 23-40, Old Irish Goat 41-52, Saanen 53-92 and Toggenburg 93-100.***

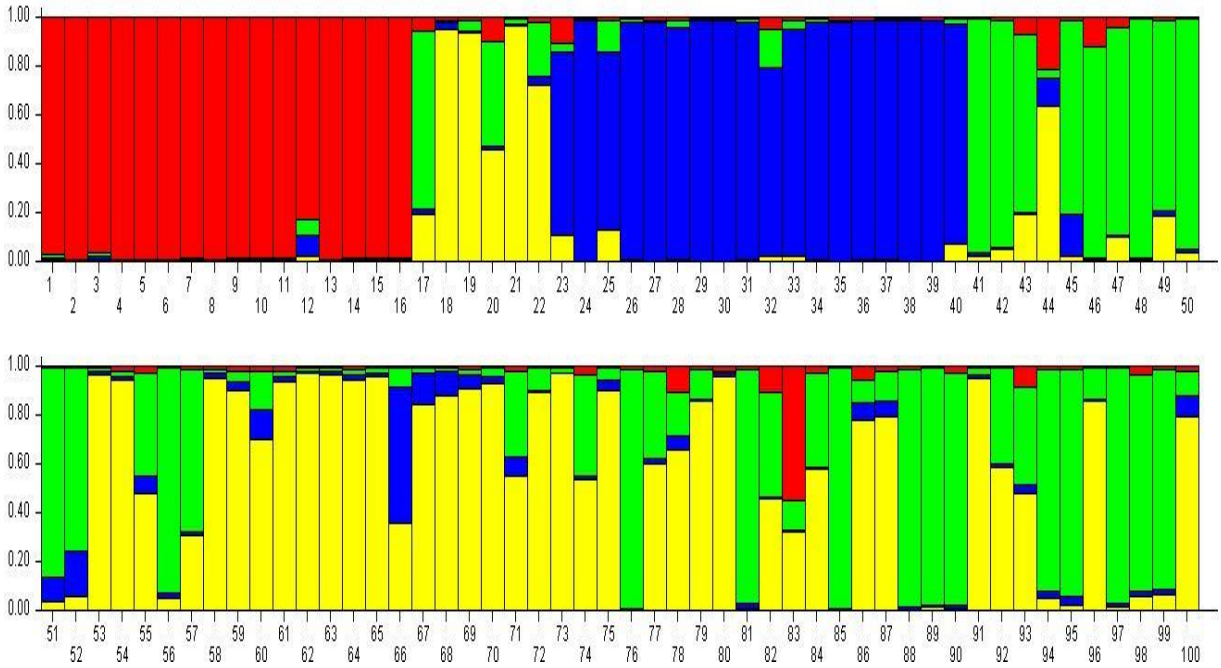


Figure 6 - Bar plot showing the population assignment percentage (Q-Values) of each individual within K=4 clusters*.
**** Sample Identifications are as follows,Anglo Nubian 1-16, British Alpine 17-22, Bilberry 23-40, Old Irish Goat 41-52, Saanen 53-92 and Toggenburg 93-100.***

3.2 Mitochondrial DNA Analysis

3.2.1 Sequence Variation, Genetic Diversity and Demographic Analysis

An aligned 598-bp fragment was constructed using both forward and reverse complement sequences for 75 samples. Sample numbers for each population are shown on Table 12, the remaining no of samples per populations were unsuitable for analysis and did not amplify when used for mitochondrial analysis. The analysed sequence contained the hyper-variable section of the Caprine mtDNA control region. Of the total 598 nucleotide sites 54 variable sites were observed which represents ≈9% of the overall fragment. The 54 variable sites contained 10 singleton variable sites and 44 parsimony informative sites. Indel analysis showed that no samples contained insertions or deletions.

Table 12 – Distribution of haplotypes per breed

Breed of Goat	Anglo Nubian	Toggenburg	Saanen	British Alpine	Old Irish Goat	Bilberry
Haplotype						
2	5					
3	10					
4				1		
5					2	
6		1			3	
7			4			
8			1			
9			2			
10			1			
11			1			
12			1		1	1
13			1			
14			2			
15			2			
16		1	1			
17			1			
18			1			
19			1			
20			1			
21		1				
22		3				
23		1				
24		1				
25						16
26				1		
27				1		
28				1		
29				1		
30				1		
31					2	
32					1	

Table 13- Np – Nucleotide positions and HAP – Haplotypes. Haplotype 1 is the reference sample (AF533441 nucleotide positions 15653-16250)

NP	15673	15677	15685	15738	15746	15750	15757	15809	15819	15831	15832	15834	15840	15841	15848	15854	15871	15885	15887	15891	15894	15909	15918	15919	15928	15948	15963	15967	15970	15971	15972	15973	15974	15976	15978	15979	15980	15981	16000	16001	16017	16019	16024	16025	16026	16035	16036	16038	16040	16041	16043	16079	16081	16148	16158	16230
HAP																																																											
1	T	T	C	T	T	G	T	T	A	C	A	G	A	G	T	A	A	G	C	C	C	A	G	T	T	T	T	T	T	A	T	T	T	T	T	C	G	G	A	C	G	C	A	G	G	C	A	T	T	T	C	G	T	C	C	C
2	*	*	*	*	C	*	*	*	G	*	*	*	*	*	*	*	*	C	*	*	*	*	A	C	*	C	*	*	C	G	C	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	C	*	*	*	*	*	A	*
3	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	C	*	*	*	G	A	*	*	*	*	*	C	*	C	*	*	C	*	*	*	*	*	*	*	*	*	*	A	T	*	*	*	*	*	*	*	*	A	*
4	*	*	*	*	*	*	*	C	*	*	*	*	*	A	*	*	*	C	*	*	*	*	A	*	*	*	*	*	*	*	C	*	*	*	*	T	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	T
5	*	*	*	*	*	*	*	C	*	*	*	*	*	A	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	T	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	C	*	*	*	C	C	G	C	*	*	*	*	*	*	*	*	*	*	A	*	*	A	*	*	*	*	*	*	*	*	*	A	*
7	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	C	*	*	*	*	G	C	*	*	*	*	*	*	A	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
8	*	*	C	*	*	*	*	C	*	*	*	G	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	G	C	*	*	*	*	*	*	*	*	*	*	T	*	*	A	*	*	*	*	*	*	*	*	*	A	*
9	*	*	*	*	*	*	*	C	*	T	G	*	*	A	*	*	*	C	*	*	*	*	A	*	*	C	*	*	*	G	C	C	*	*	*	T	*	*	*	*	*	*	*	*	A	*	G	*	*	*	*	C	*	A	*
10	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	G	C	*	*	*	*	A	*	G	*	A	*	*	*	*	A	*	*	C	*	*	T	A	*	*	A	*
11	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	*	C	*	A	*	*	G	*	*	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
12	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	C	*	*	T	*	*	*	*	*	*	*	*	*	C	C	*	C	*	T	*	*	*	*	T	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
13	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	C	*	T	T	*	A	*	*	C	*	*	*	G	*	C	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
14	*	*	*	*	*	*	*	*	T	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	C	*	*	*	G	*	*	*	*	C	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	C	*	A	*
15	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	C	*	*	*	*	A	*	*	*	*	*	*	G	C	*	*	*	*	A	*	*	A	*	*	*	*	*	A	*	*	C	*	*	T	*	*	*	A	*
16	*	*	*	*	A	*	C	*	*	*	*	G	*	*	*	*	*	C	*	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	*	*	*	*	*	*	*	T	*	*	A	*	*	*	*	*	T	*	*	*	A	*
17	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	G	T	*	*	*	*	*	*	*	C	*	*	*	G	C	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	T	A	*
18	*	*	T	*	*	A	*	C	*	*	*	*	G	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	G	C	*	*	*	*	*	*	*	*	*	*	T	*	*	A	*	*	*	*	*	T	*	*	*	A	*
19	*	*	*	*	*	*	*	C	*	*	*	*	*	A	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	G	C	*	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
20	*	*	*	*	*	C	C	*	*	*	A	*	*	*	*	*	G	C	*	*	*	*	*	*	*	*	*	*	*	G	C	*	C	*	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
21	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	*	*	C	*	C	*	G	C	C	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
22	*	C	*	*	*	*	*	C	*	*	*	*	*	C	*	*	*	C	T	*	*	*	A	*	*	*	*	C	*	G	C	*	*	*	*	*	*	A	*	*	*	*	G	*	A	*	*	*	*	C	T	*	*	*	A	*
23	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	*	C	*	A	*	*	*	C	*	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
24	*	*	*	*	*	*	*	C	G	*	*	*	*	*	*	*	*	C	*	T	*	*	A	*	C	C	A	*	*	*	C	*	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
25	*	*	*	C	*	*	*	G	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	C	*	*	*	*	C	G	C	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
26	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	*	*	C	*	C	*	G	C	C	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	T
27	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	T	T	*	A	C	*	C	*	*	*	G	*	C	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	T
28	T	C	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	T	*	*	*	A	*	*	C	*	C	*	G	C	*	*	*	*	T	*	A	*	*	*	*	G	A	A	*	*	*	*	*	C	T	*	*	*	A	T
29	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	T	T	*	A	*	*	C	*	*	*	G	*	C	*	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	T
30	C	*	*	*	*	*	*	C	*	*	*	*	G	*	*	*	*	C	*	*	*	*	A	*	*	*	A	*	*	*	*	C	C	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	T
31	C	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	*	*	*	A	*	*	*	A	*	*	*	*	C	C	*	*	T	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	A	*
32	*	*	*	*	A	*	C	*	*	*	*	G	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	T	*	*	A	*	*	*	*	*	T	*	*	*	A	*

DnaSP v5 was used to carry out genetic diversity analysis of the mtDNA partial D-loop sequences. The number of variable sites (VS) and mean nucleotide diversity (π) (Tajima, 1993) was calculated for each population. VS ranged from 10 in the Anglo Nubian goats to 39 in the Saanen goats. The mean nucleotide diversity ranged from 0.00231 for the Bilberry goats to the Toggenburg goats 0.01526

Demographic history analysis was carried out using ARLEQUIN v3.0 to analyse Fu's *Fs* (Fu,1997) statistics, Tajima's *D* (Tajima's, 1989) statistics and DnaSP v5 was used to calculate Mismatch Distributions (Slatkin and Hudson, 1991). The Anglo Nubian, Toggenburg, Old Irish Goats and the Bilberry goats all had positive Fs values and the Anglo Nubian, Toggenburg and the Old Irish goats have positive Tajima's D values.

3.2.2 Haplotype Diversity

Each population was analysed with DNASp ver5 for haplotype diversity. The Bilberry goats had the lowest haplotype diversity at 0.12 with only 2 different haplotypes present in the 17 samples analyzed. They contain 16 haplotypes which are identical and 1 haplotype that is different. The British Alpine goats had 100% diversity for all the 6 samples used with 6 different haplotypes present in the sample set used. Thirty one different haplotypes were discovered within the 75 samples analysed. Haplotype 24 showed the most sequences at 16 samples which belonged solely to the Bilberry goat population. Eighteen haplotypes were only represented by one sequence throughout all the populations. The Anglo Nubian goats and the Bilberry goats showed the lowest number of haplotypes at 2 and the Saanen goats showed the highest amount of haplotypes at 14.

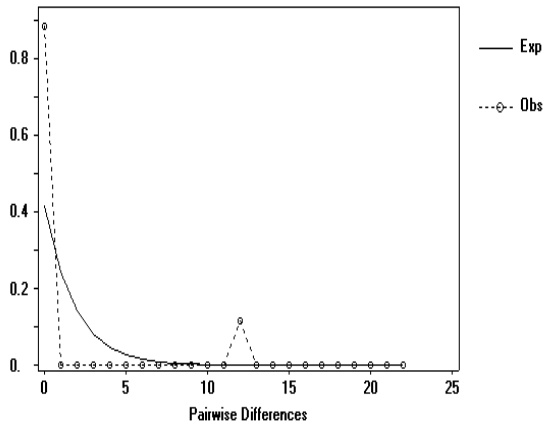
Table 14 – Sample size (n), Number of haplotypes (H), Haplotype diversity (h), Nucleotide diversity (π), Fu's F_s, Tajima's D for the six different populations studied.

Population	n	VS	π	Fu F's Values	Tajima's D Values	H	h
Anglo Nubian	15	10	0.00779	9.05	2.08716	2	0.476
Toggenburg	8	24	0.01526	0.605	0.03702	6	0.893
Saanen	20	39	0.01461	0.75	-2.22953	14	0.953
British Alpine	6	19	0.01266	-1.37	-0.4404	6	1
Old Irish Goat	9	20	0.01237	2.25	0.13153	5	0.861
Bilberry	17	12	0.00231	3.77	-2.2621	2	0.118

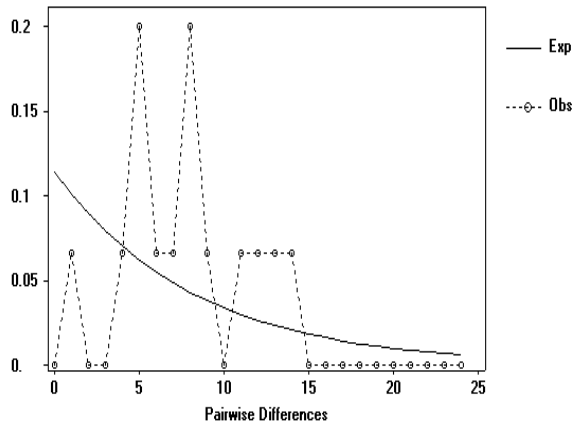
Mismatch distributions were plotted against each other to compare population distributions between observed and expected mismatch distributions. All populations except for the Bilberry and the Anglo Nubian goats showed multi modal distributions when graphed against the expected distribution. Figure 7 shows the expected and observed mismatch distributions for each population.

3.2.3 Haplogroups

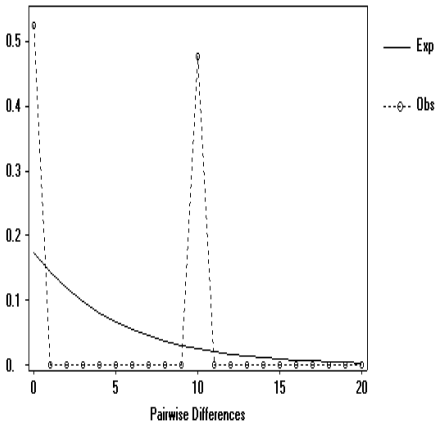
When all the haplotypes that have been sequenced in this study are compared against the 22 reference samples which are the samples representing Haplogroup A,B,C,D,F and G, it is found that the thirty one different haplotypes all belong to haplogroup A. The standard criteria set out by Naderi et al., 2007 is that the haplotypes should differ from less 20 mismatches within the selected groups. A neighbour joining tree was constructed for the 31 different haplotypes and the 22 reference samples and it is outlined below in Figure 8



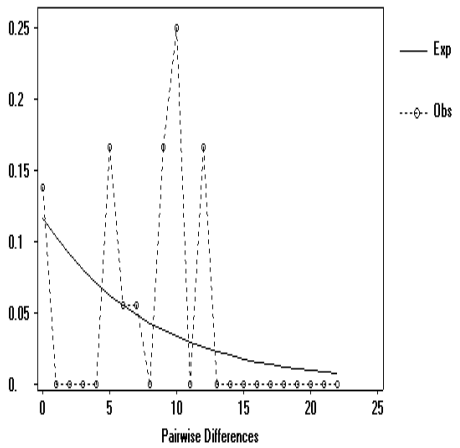
Bilberry



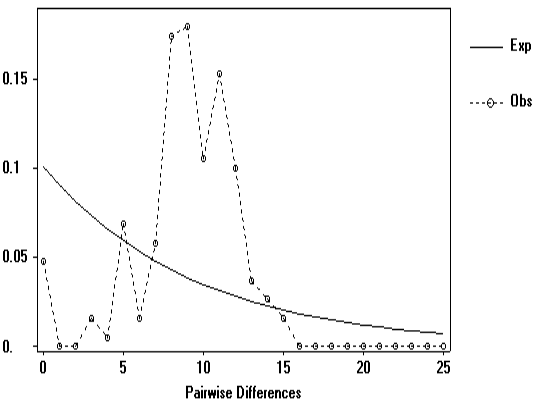
British Alpine



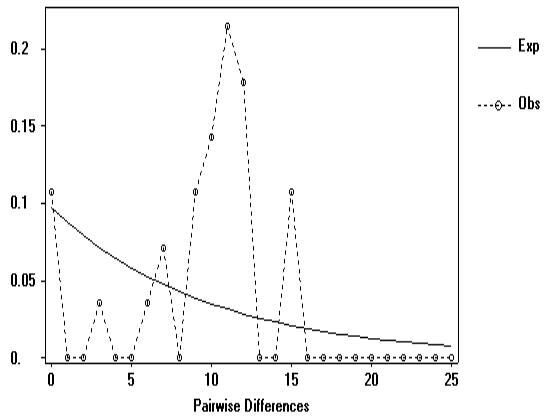
Anglo Nubian



OIG



Saanen



Toggenburg

Figure 7 – Mismatch Distributions for each population

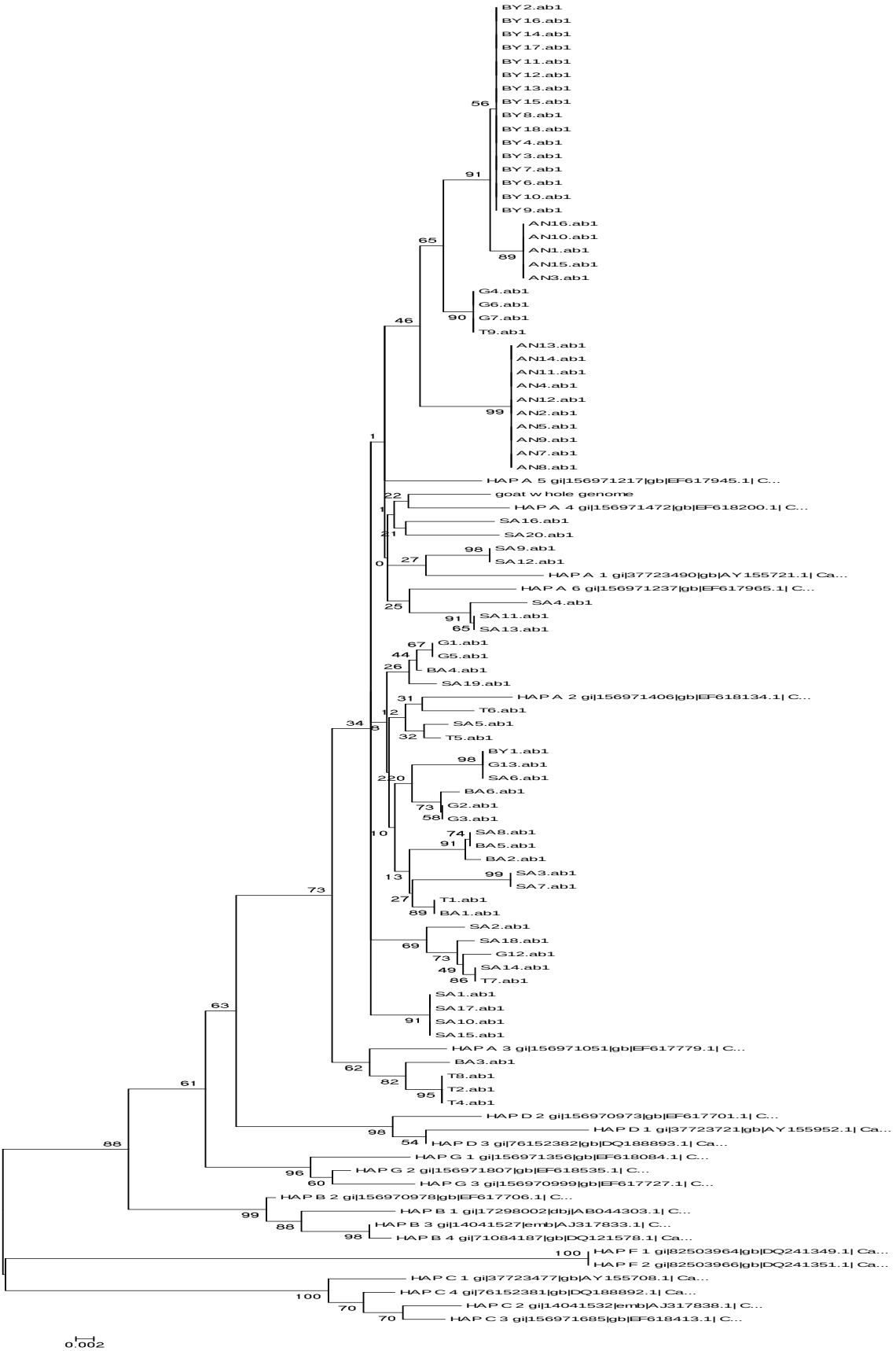

BY2.ab1
BY16.ab1
BY14.ab1
BY17.ab1
BY11.ab1
BY12.ab1
BY13.ab1
BY15.ab1
BY8.ab1
BY18.ab1
BY4.ab1
BY3.ab1
BY7.ab1
BY6.ab1
BY10.ab1
BY9.ab1
AN16.ab1
AN10.ab1
AN1.ab1
AN15.ab1
AN3.ab1
G4.ab1
G6.ab1
G7.ab1
T9.ab1
AN13.ab1
AN14.ab1
AN11.ab1
AN4.ab1
AN12.ab1
AN2.ab1
AN5.ab1
AN9.ab1
AN7.ab1
AN8.ab1
HAP A 5 gi|156971217|gb|EF617945.1| C...
goat w hole genome
HAP A 4 gi|156971472|gb|EF618200.1| C...
SA16.ab1
SA20.ab1
SA9.ab1
SA12.ab1
HAP A 1 gi|37723490|gb|AY155721.1| Ca...
HAP A 6 gi|156971237|gb|EF617965.1| C...
SA4.ab1
SA11.ab1
SA13.ab1
G1.ab1
G5.ab1
BA4.ab1
SA19.ab1
HAP A 2 gi|156971406|gb|EF618134.1| C...
T6.ab1
SA5.ab1
T5.ab1
BY1.ab1
G13.ab1
SA6.ab1
BA6.ab1
G2.ab1
G3.ab1
SA8.ab1
BA5.ab1
BA2.ab1
SA3.ab1
SA7.ab1
T1.ab1
BA1.ab1
SA2.ab1
SA18.ab1
G12.ab1
SA14.ab1
T7.ab1
SA1.ab1
SA17.ab1
SA10.ab1
SA15.ab1
HAP A 3 gi|156971051|gb|EF617779.1| C...
BA3.ab1
T8.ab1
T2.ab1
T4.ab1
HAP D 2 gi|156970973|gb|EF617701.1| C...
HAP D 1 gi|37723721|gb|AY155952.1| Ca...
HAP D 3 gi|76152382|gb|DQ188893.1| Ca...
HAP G 1 gi|156971356|gb|EF618084.1| C...
HAP G 2 gi|156971807|gb|EF618535.1| C...
HAP G 3 gi|156970999|gb|EF617727.1| C...
HAP B 2 gi|156970978|gb|EF617706.1| C...
HAP B 1 gi|17298002|dbj|AB044303.1| C...
HAP B 3 gi|14041527|emb|AJ317833.1| C...
HAP B 4 gi|71084187|gb|DQ121578.1| Ca...
HAP F 1 gi|82503964|gb|DQ241349.1| Ca...
HAP F 2 gi|82503966|gb|DQ241351.1| Ca...
HAP C 1 gi|37723477|gb|AY155708.1| Ca...
HAP C 4 gi|76152381|gb|DQ188892.1| Ca...
HAP C 2 gi|14041532|emb|AJ317838.1| C...
HAP C 3 gi|156971685|gb|EF618413.1| C...
0.002

Figure 8 - Neighbour joining tree depicting evolutionary relationships of 98 taxa.

4.0 Discussion

This study represents the first results on characterization of genetic variability detected through microsatellite markers in the two native breeds of goats in Ireland and also four other breeds of goats present in Ireland. This study compared two native breeds of goats to four other domestic breeds of goats that are bred in Ireland. The Bilberry goats have been an enclosed group of goats for over 200 hundred years in an area known as the Bilberry Rock in Waterford City. The herd has had no known interference with other breeds of goats in that time. The Old Irish Goats that are represented in this study are goats that have been chosen from a large group of goats in the Burren that phenotypically represent the breed. Interference from other breeds of goats with the Old Irish Goat is highly possible as the Burren has a high level of goats being abandoned in the region.

4.1 Microsatellite Discussion

All 10 microsatellite loci studied were polymorphic with polymorphic information content of over 0.4 for all the microsatellites making them useful for genetic diversity studies (Bruno de Sousa et al., 2010). The level of genetic diversity found in the Bilberry goats is low with He values for all the loci at 0.51 the Anglo Nubian and the Bilberry goats showed low levels of genetic diversity with He values of 0.45 and 0.51 and also low levels of alleles 2.8 and 3.4. These values are lower for that seen in Swiss breeds with He values of 0.6 (Saitbekova et al., 1999) and Chinese breeds with He values of 0.82 (Qi et al., 2009). All the other breeds of goats studied are close to the mean of He of 0.69 reported by Canon et al., 2006. The Mean Number of alleles ranged from 2.8 to 6.7 which is lower in value than in other studies which have ranged from 5.9 in goat breeds from the Canary islands (Martinez et al., 2006) to 10.2 for Chinese goats (Qi et al., 2009). Low number of alleles per locus could be a result of sample size or past bottlenecks which are known to affect more allelic richness than the level of genetic variability (Luikart and Cornuet, 1998). High levels of heterozygosity should equal to high levels of

diversity. The two native breeds included in this study showed similar levels of expected heterozygosity with the Old Irish Goats with a He value of 0.66 and the Bilberry goats with a He value of 0.511. These values of expected heterozygosity are compatible with values of 0.68 for Serpentina breed from Portugal (Oliveira, et al., 2010), 0.69 observed in Portuguese goat breeds (Bruno-de-Sousa et al., 2010). Saitbekova et al., 1999 found that in their study on diversity that heterozygosity was higher in domestic goat breeds than in wild goats in their study of Swiss goats with He values ranging from 0.51 to 0.58 for domestic herds and from 0.17 to 0.19 for the wild species of bezoar and ibex goats. This study showed He values of 0.5 to 0.7 for domestic breeds and the same values of 0.5 and 0.7 for the wild native goats which is relatively higher than what was reported in Ibex and Bezoar wild goats in Switzerland. The low values shown for the Ibex and the Bezoar goat's shows that they had very little introgression with other goat breeds over time.

The observed heterozygosity for both the native goats showed large differences. The Bilberry goat showed a difference of 0.056 against the Anglo Nubian and 0.293 against the Toggenburg goat. The Old Irish goat showed a difference of 0.148 against the Anglo Nubian and 0.201 against the Toggenburg goat. The Bilberry goats showed lower levels of diversity than the Old Irish Goat which would indicate that the population has not had any introgression from outside populations due to the isolated region of Waterford they have come from. The Saanen population was found to have high levels shown for mean no of alleles, observed heterozygosity and expected heterozygosity. As the Saanen goats are the largest breed of goat around Europe this would explain why the breed shows a large amount of genetic diversity with a larger gene pool being available to breeders to help keep the breed diverse. While the Anglo Nubian goats used in this project show the least values for genetic diversity the selection of goats might not have been the most diverse group of goats being bred in Ireland and sampling from a larger herd might help these values.

The Anglo Nubian and the Bilberry goats showed the lowest level of genetic diversity with low levels of heterozygosity and low mean number of alleles. This could indicate that both these populations have had a closed population with little or no introgression with other breeds. Low levels of allelic richness were noted in these breeds also which is more sensitive to population numbers than the other two measures. The Anglo Nubian goats also showed the lowest allelic richness of the breeds that were selected showing that the selection of goats have very little difference in the gene pool they come from. Out of the two native breeds the Old Irish Goat showed a higher level of allelic richness than the Bilberry goats although being an enclosed herd the low allelic richness would support the fact that they have not had any introgression with any other goat breeds.

Genetic variability among breeds were accounted for by differences among the breeds using $F_{st.}$ Low levels of F_{st} observed in most studies with goats has been attributed to the high mobility of the species across different regions which would have provided the opportunity for some gene flow among populations, and result in their reduced differentiation. (Luikart et al., 2001). The British Alpine, the Toggenburg and the Saanen goats were not significantly different from each other with the lowest levels among the pairing of the British Alpine and the Saanen goats. The Anglo Nubian goat and the Bilberry goat showed the highest levels of variation between the two populations with the Anglo Nubian goats showing the highest values of genetic variability against all other breeds. This was further supported by the clustering in the factorial correspondence analysis and the principal component analysis plot. The Anglo Nubian goat shows a distinct clustering with no admixture with any of the other breeds with the Bilberry goats showing the second best isolation in the clustering and the pca plot. The Saanen, the Toggenburg and the British Alpine all plot together in both plots showing a low level of differentiation based on allele sharing distances.

The measure of Wrights (1965) Fst estimator allows comparisons to be made between breeds to estimate the genetic variation between the breeds. The Anglo Nubian goats showed the highest

values of variation against all the other breeds of goats with values ranging from 0.24 – 0.36 (24%-36%) thus variation within the selection of Anglo Nubian goats is 64%-76%.

The Old Irish Goat showed values of variation of 27% against the Anglo Nubian goats and showed the least variation from the Saanen goat breed at 6%.This would lead to the assumption that some time in the past the Saanen goats breed was bred into the Old Irish Goat breed.

The Bilberry goats showed the highest variance with the Anglo Nubian goats with a value of 36% and the lowest with the Saanen goats with a value of 10%. The number of goats sampled for each population might have affected the Fst estimation and also low polymorphism within the breeds themselves also can affect the estimation.

Other microsatellite studies of goat breeds have showed low FST values also and these values have been explained by the goat's high level of mobility across the different regions of the world which would have allowed gene flow among populations and result in reduced differentiation (Luikart et al 2001; Naderi et al., 2007)

The variation found using the Fst estimations is further backed up by the factorial correspondence analysis (FCA) which graphically separates the populations from each other. The Anglo Nubian goats show the most differentiation but the Old Irish Goats and in particular the Bilberry goats show distinct variation from other breeds. The lack of differentiation of the Saanen, British Alpine and the Toggenburg goats could indicate that these goat breeds have an admixture between their genetic resources in their histories. Before herd books for these breeds were established breeding between all breeds of goats was common and is continued to this day to improve the qualities needed by goats in new and emerging markets. STRUCTURE also backed up these findings of breed admixture. STRUCTURE showed that there were most likely 3 or 4 populations in the data supplied. The assumption that the six breeds were distinct breeds with no admixture between breeds was incorrect. As the graph on figure 4 shows that

STRUCTURE shows that the number is more than likely 3 or 4 populations as they show the least standard deviation away from the likelihood that the populations belong to these clusters. In figures 5 and 6 show the bar plots for which each individual is assigned into a population. The Anglo Nubian goats show very little admixture with other breeds and also the Bilberry goats show that they also have very little admixture with other breeds. The British Alpine and the Old Irish goats show the next differentiation and then the Saanen and the Toggenburg just look like an admixture. STRUCTURE and FCA support each other in their findings that the Bilberrys and the Anglo Nubian show genetic differentiation from the other breeds sampled.

As the Old Irish goat showed the only positive mean value for F_{is} which shows that the population has undergone some inbreeding among its population this could be influenced by the fact that a group of goats that are phenotypically representative of the breed have been taken into captivity to try and maintain the breed which has limited the gene pool that the goats can breed from. The Toggenburg goats showed the highest negative value for F_{is} which is indicative of outbreeding and this is also evident with its low level of differentiation from the other commercial breeds.

Fis is the value by which inbreeding is measured. Negative values of Fis indicate excessive heterozygotes and positive values of Fis indicate a deficiency in heterozygotes. Positive values show that inbreeding has occurred within the population. The Old Irish Goats was the only breed to show a positive overall value for Fis at 0.029. Although the value is not statistically significant $P<0.05$ this selection of goats for a closed herd of Old Irish Goats has a low level of heterozygosity and going forward with a breeding program with these goats this value is only going to get higher as the herd is starting with a low level of heterozygosity.

All breeds were shown to be in Hardy Weinberg Equilibrium. To be in HWE populations have allele and genotype frequencies that have remained constant thus they are in equilibrium and have not been effected by non-random mating, mutations, selection, random genetic drift, gene

flow and meiotic drive. Deviations from HWE can suggest a level of inbreeding within populations. Within such small select populations it is hard to believe that all these factors have been adhered to but the breeding that has been carried out within these breeds have maintain HWE thus maintaining sufficient allele and gene frequencies.

4.2 Mitochondrial discussion

Using the standard criteria for the definition of mitochondrial haplogroups set out by Naderi et al., 2007 all the goats samples that were sequenced in this study belonged to Haplogroup A. Haplogroup A represents 91 % of the goats sampled worldwide (Naderi et al., 2007) thus demonstrating the weak phylogeographic nature of goats. This weak phylogeographically structure has been explained by the high rate of mobility of goats in relation to human migration and commercial trade in the past (Luikart et al., 2001).Another previous feature of other goat mitochondrial studies show that as a breed they have multiple maternal origins (Luikart et al., 2001). In this study the Bilberry goats that were sampled showed two different haplotypes with sixteen of the seventeen amplified showing the same haplotype. A BLAST search of this haplotype reveals that it has previously been amplified in a study of Chinese native goats (Xu et al., 2001). The one other haplotype from an individual Bilberry goat was also amplified in the Old Irish Goat and the Saanen goat which suggests that there has been introgression with another breed sometime along in the Bilberry history.

The Old Irish Goat Breed showed five different haplotypes for the nine samples that were amplified showing 86 % haplotype diversity. As the Old Irish Goat herd has past history of introgression when domestic stock were allowed to mix with the wild herds this would explain these different haplotypes being present. Three of the nine haplotypes were shared with the Toggenburg breed and one of the haplotypes was shared with the Saanen breed. The Anglo Nubian goat did not share any of its two haplotypes with any other breed of goat thus inferring that this breed has become very pure in its breed since the initial breed set up with crossing native goats in Britain with four imported male Nubian Goats. The British Alpine goat showed

100% haplotype diversity with six different haplotypes present in the goats sampled. The six different haplotypes were not amplified in any other goats in this study. The Saanen showed 95% haplotype diversity with fourteen different haplotypes present in the twenty samples that were amplified. Only one of these haplotypes are shared with the Toggenburg goats which is a little surprising as both the goat breeds originated in Switzerland although the Saanen goats came via Holland into England.

The neighbour joining tree in Figure 8 shows that the Bilberry goats and the Anglo Nubian goats show levels of genetic independence in comparison with the other breeds that were sampled. This backs up the microsatellite findings in the FCA plot and the STRUCTURE analysis. Breed admixture was shown between the Old Irish Goats, the Saanens, the Toggenburgs and the British Alpine in the neighbour joining tree.

The Bilberry goats showed low nucleotide diversity at 0.0023 and also the breed showed negative values for Tajima's D (-2.2621). Both these values indicate low frequency polymorphisms relative to expectations which indicate that the population is undergoing an expansion after a bottleneck event. This evidence backs up the history of this breed as in 1990 there was five males and two females left in the herd and the area they had inhabited had been reduced severely. Both of these values indicate low frequency polymorphisms relative to expectations which indicate that the populations underwent an expansion after a bottleneck event. This is further backed up by the mismatch distributions showing a unimodal pattern.

The Old Irish Goat showed low nucleotide diversity (0.01237) with positive Tajima D values 0.1315 indicating a decreasing population size and mismatch distributions showing multimodal distributions which shows that the population is at a demographic equilibrium. This is further supported by the history of the herd as a select group has now been separated in order to preserve the breed.

The British Alpine breed displayed a negative Fu F'*s* values -1.37. This is an indication that this population is undergoing a population demographic expansion. With negative Tajima's D values observed it might suggest that this population has undergone a bottleneck in the past prior to this growth. The Saanen goat population had negative values for these two measures also which suggests that this population does not conform to expectations of neutrality and may have undergone expansions or bottlenecking of their populations also.

4.3 Conclusions

Of the two native goat breeds studied the Bilberry goats showed the most genetic independence from the other goat breeds present in Ireland. The Old Irish Goat showed some levels of independence but overall the selection showed admixture with the Saanen, the British Alpine and the Toggenburg goats.

Genetic differentiation estimators based on microsatellite data showed significant values for the Bilberry goats from the other goat breeds studied. The Old Irish Goats showed to a lesser degree differentiation.

The Bilberry goats showed high levels of independence from the other goats sampled both at a microsatellite and a mitochondrial level. The Bilberry goats showed very little introgression with other goat breeds

The Saanen, the Toggenburg and the British Alpine goats showed admixture between each population both at a microsatellite and a mitochondrial level. The Old Irish goat showed admixture also with these breeds.

Clustering suggests high levels of breed purity in the Anglo Nubian goats sampled and also in the Bilberry goats that were sampled. The Old Irish goat showed clustering with the Saanens, the Toggenburgs and the British Alpine.

The Old Irish Goats and the Bilberry goats showed no significant levels of inbreeding.

The Bilberry goat population showed levels of genetic isolation and genetic uniqueness at both a microsatellite and a mitochondrial level

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