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Genetic variation within the Icelandic goat breed

Assessment using population data and DNA analysis

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Clarification of contribution

I hereby declare that the writing of the following thesis is my work, done under the supervision and with the assistance of my advisors and members of my master committee, Dr. Jón Hallsteinn Hallsson and Þorvaldur Kristjánsson MSc.

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Birna Kristín Baldursdóttir

Abstract

The Icelandic goat breed is a very small closed population consisting of only about 500-600 animals kept in 45 herds. The breed is believed to have originated from Norway during the settlement of Iceland in the years 874-930 AD. Several population bottlenecks are known to have occurred and the population has at least twice declined to under 100 animals. In the present study the genetic diversity within the Icelandic goat population is estimated on the base of both pedigree information and DNA analysis. The estimate is based on three approaches. Firstly, pedigree information is used to calculate the rate of inbreeding, pedigree completeness, generation interval, effective population size, highest contributing ancestors and relationship within and between areas. Secondly, microsatellite markers were used for genotyping and various diversity measures were calculated as well as the effective population size. Thirdly, parts of the mtDNA D-loop were sequenced in order to estimate the genetic diversity and structure of the population.

Genetic diversity of livestock is an important factor in animal breeding and it is the basis for all genetic adaptability. The major concern of Icelandic goat breeders is that the breed might become extinct, due to a small population size, extensive fragmentation and high levels of inbreeding. The findings presented here confirm their concern. The genetic diversity of the population is shown to be very low and the inbreeding levels high. Results from pedigree data show that the estimated annual rate of inbreeding is around 3% and the generation interval 3.5 years, corresponding to an increase in inbreeding of 9.9% per generation and the average inbreeding within the population in the year 2006 was 15.9% (PEC5 $\geq$ 0.24). The estimated effective population size is 5.1 animals. The two most influential ancestors in the years 2002 and 2006 contributed 9.5% and 16.5% of the genetic material, respectively. Relationship calculations showed that there is relatively higher relationship within areas than between areas. Microsatellite analysis revealed a mean number of observed alleles per locus of 1.8, ranging from 1 to 4 for individual markers, six markers were monomorphic. The overall mean observed heterozygosity of 0.178 was lower than the mean overall expected heterozygosity of 0.185. The effective population size was estimated to be 4.1-8.8 individuals. Results from the mtDNA D-loop sequencing showed three haplotypes all representing the most common goat haplogroup A.

Altogether, these results confirm that the Icelandic goat population is in a critical state with extremely low genetic diversity.

Ágrip

Íslenski geitastofninn er lítill lokaður erfðahópur sem telur á milli 500-600 dýr í 45 hjörðum. Talið er að landnámsmenn hafi flutt geitur með sér frá Noregi um 870-930. Vitað er að stofninn hefur gengið í gegnum nokkra flöskuhálsa og tvisvar hefur stofninn farið niður fyrir hundrað dýr. Í rannsókn þessari var erfðafjölbreytileiki innan íslenska geitastofnsins metinn bæði með ætternisgögnum og DNA greiningu. Matið byggðist á þremur aðferðum. Í fyrsta lagi voru ætternisgögn notuð til að reikna skyldleikaræktaraukningu, ættarstuðul, ættliðabil, virka stofnstærð, erfðaframlag helstu ættfeðra og mæðra ásamt skyldleika milli og innan svæða. Í öðru lagi voru örtungl notuð til að meta fjölda arfblendinna einstaklinga og virka stofnstærð út frá erfðamörkum. Í þriðja lagi var hluti hvatberaerfðamengisins, D-lykkjan, raðgreind til að meta erfðafjölbreytileika og skiptingu stofnsins.

Erfðafjölbreytileiki er mikilvægur þáttur í kynbótum og grunnur þróunar og aðlögunar lífvera. Helsta áhyggjuefni íslenskra geitfjárræktenda hefur verið að stofninn væri í mikilli útrýmingarhættu vegna lítillar stofnstærðar, mikillar einangrunar hópa innan stofnsins og þar af leiðandi mikillar skyldleikaræktar. Niðurstöður þær sem kynntar eru hér staðfesta að staða stofnsins er afar viðkvæm þar sem erfðafjölbreytileiki er lítill og skyldleikarækt mikil. Niðurstöður reiknaðar útfrá ætternisgögnum sýndu að árleg aukning í skyldleikarækt er 3%, ættliðabil 3,5 ár sem svarar til 9,9% aukningar í skyldleikarækt í hverri kynslóð og meðal skyldleikaræktarstuðull árið 2006 var 15,9% (ættarstuðull $\geq$ 0,24). Virk stofnstærð var metin 5,1 dýr. Erfðaframlag tveggja áhrifamestu ættfeðra og mæðra árin 2002 og 2006 var 9,5% og 16,5%. Skyldleiki geita innan svæða reyndist í flestum tilfellum vera meiri en skyldleiki milli svæða. Niðurstöður örtunglagreiningar sýndu að meðalfjöldi samsæta í hverju sæti (MNA) var 1,8 og lágu gildi fyrir einstök örtungl á bilinu 1 til 4, sex samsætur voru einsleitar. Meðal fundin (H_O) og væntanleg (H_E) arfblendni var 0,178 og 0,185. Virk stofnstærð var metin sem 4,1-8,8 einstaklingar. Niðurstöður raðgreiningar á D-lykkju hvatberaerfðamengisins leiddu í ljós að þrjár mismunandi setraðir voru til staðar sem allar tilheyra hópi A sem er algengasti setraðahópur hjá geitum.

Samantekið sýna þessar niðurstöður að staða íslenska geitastofnsins er alvarleg og afar viðkvæm, þar sem erfðafjölbreytileiki er mjög takmarkaður.

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Table of contents

List of tables

List of figures

List of abbreviations

AI: Artificial insemination

D-loop: Mitochondrial DNA displacement loop

EVA: EVolutionary Algorithm for mate selection

F: Inbreeding coefficient

F_{IS}: Within population inbreeding coefficient

FAO: Food and Agricultural Organization of the United Nations

ΔF: Change in inbreeding per generation

ΔF_y: Change in inbreeding per year

IAM: Infinite allele model

IBD: Identical by descent

IIS: Identical in state

ISAG: International Society for Animal Genetics

H_O: Observed heterozygosity

H_E: Expected heterozygosity

HWE: Hardy-Weinberg equilibrium

L: Generation interval

MNA: Mean number of alleles

N_e: Effective population size

N_{OA}: Number of observed alleles

NGH: Nordisk Genbank Husdyr

PCR: Polymerase chain reaction

PIC: Polymorphism information content

PEC: Pedigree completeness index

SMM: Stepwise mutation model

SNP: Single nucleotide polymorphism

TPM: Two-phased model of mutation

QTL: Quantitative trait loci

1 Introduction

1.1 Genetic diversity and conservation

Genetic diversity is defined as the sum of genetic differences in multiple loci among individuals in a population, and is most readily reflected in the phenotypic variation seen in many populations. Genetic diversity is a valuable asset as the adaptability of a population, that is the population's ability to adapt to changes, depends on it (Woolliams, Berg, Mäki-Tanila, Meuwissen & Fimland, 2005). It is well known that species can face great environmental changes over time, such as in climate, pollution and in diseases, and genetic diversity is required for populations to adapt to these changes (Frankham, Ballou & Briscoe, 2002). The long term consequences of intense selection, be that due to changing market demands or a drive towards increased economic returns, are of great concern for many populations, both those under selection and those that are considered unfavorable for the market (Woolliams *et al.*, 2005). This is not least due to the fact that intense selection leads to inbreeding and inbreeding has been shown to increase the risk of extinction in captive populations (Brook, Tonkyn, O´Grady & Frankham, 2002; Frankham *et al.*, 2002). Loss of genetic diversity is often associated with inbreeding and reduction in reproductive fitness (Frankham *et al.*, 2002; Willi, Buskirk & Hoffmann, 2006) and although there has been some disagreement regarding the importance of genetic factors in population extinctions (Frankham *et al.*, 2002) it has been established that most species do not become extinct before genetic factors negatively affect them (Spielman, Brook & Frankham, 2004). This has been demonstrated to apply to species (Spielman *et al.*, 2004) and there is no reason that it could not apply to individual populations within species as well.

Many plant and animal species around the world are at risk of extinction, largely due to human activities (Lande, 1998). During the past fifteen years 300 of the 6000 farm animal breeds identified by the Food and Agricultural Organization (FAO) have become extinct and 1350 breeds are at risk of extinction in the near future. During this period, fourteen European goat breeds have become extinct (Taberlet *et al.*, 2008). Goats are one of the worlds most adaptable and widespread livestock species, and are one of the main economic recourses in many developing countries and their economic importance is growing in western countries (Luikart *et al.*, 2001). Fortunately, the market demand, at least in some parts of the world, are changing and the demand for specialty products (niche-products) is growing. This gives

breeders of original/rare breeds an opportunity to expand the stock and preserve its genetic diversity (Eyþórsdóttir, Tómasson & Helgadóttir, 2001).

One of the primary goals in the management of animal populations is to maintain their genetic diversity at a high level and their inbreeding at a low level (Fernández, Villanueva, Pong-Wong & Toro, 2005). To estimate the future breeding potential of a livestock breed it is necessary to characterize the genetic structure and estimate the level of genetic diversity within the breed. For this purpose pedigree information (Caballero & Toro, 2000; Cervantes, Goyache, Molina, Valera & Gutiérrez, 2008) and genetic material (Saitbekova, Giallard, Obexer-Ruff & Dolf, 1999; Dasmahapatra, Lacy & Amos, 2008) are used. From pedigree information, the level of inbreeding and relationships in the population can be estimated and furthermore the effective population size, which is regarded as a good indicator of the change in genetic variability over a long time (Boichard, Maignel & Verrier, 1997). When using pedigree information to calculate inbreeding related parameters the outcome is dependent on the completeness of this information, and changes in inbreeding due to different breeding practices and bottlenecks are not immediately perceivable using this approach (Boichard *et al.*, 1997). Therefore, parameters based on the probability of gene origin from different herds, founders and ancestors have been proposed as complementary indicators, as they provide more information about changes occurring in the population over a short period of time (Boichard *et al.*, 1997). From genetic material, microsatellite markers and mitochondrial DNA displacement loop (D-loop) are commonly used to describe the genetic polymorphism, genetic distance and geographical origin of the domestic goat (Saitbekova *et al.*, 1999; Luikart *et al.*, 2001; Naderi *et al.*, 2007; Agha *et al.*, 2008). The advance of molecular genetics in resent decades has made it possible to differentiate the relationship between pairs of individuals, which according to the pedigree have the same relationship.

The study presented here deals with genetic diversity within the Icelandic goat population using pedigree information and methods of molecular genetics. To introduce the matter, findings of related research are discussed and the Icelandic goat breed described, including its development and the current status of the breed.

1.2 Genetic diversity within small populations

The genetic structure of a population is determined by the number and frequencies of alleles and the forces affecting them – mutation, migration, selection and random drift, but also by the population mating system. Random mating may take place where individuals in a

population have an equal chance of mating with any other individuals. However, most populations deviate from random mating and when mates are chosen that are more closely related than individuals chosen at random it results in inbreeding (Hallerman, 2003). Small populations that remain isolated for many generations can face serious threats and are more vulnerable to both genetic and environmental changes. Small populations become inbred at a faster rate than large populations because mating of related individuals is unavoidable, resulting in inbreeding and decreased genetic diversity (Falconer & Mackey, 1996; Frankham *et al.*, 2002; Willi *et al.*, 2006). Genetic diversity depends on many other factors than population size, such as the number of loci affecting a trait, dominance and/or epistasis (interaction of genes within and between loci), the effects and fixation probabilities of new mutations, selection intensity and selection mode. Stress, whether it is environmental or genetic, can determine whether a population adapts to a changing environment or declines to extinction. Environmental stress refers to how individual fitness can decline due to ecological factors, while genetic stress is caused by inbreeding depression, genetic load or reproductive incompatibility (Willi *et al.*, 2006). Studies of small populations, using *Drosophila melanogaster* as a model organism, with different levels of inbreeding and high temperature and ethanol as stress factors, showed that environmental stress becomes significantly greater with higher inbreeding levels. Although these two factors are not independent they can act synergistically (Bijlsma, Bundgaard & Boerema, 2001).

1.2.1 Inbreeding

Inbreeding is the mating of related individuals, and results in some loci bearing alleles that are identical by descent (IBD). This occurs because alleles from one common ancestor may flow through multiple offspring. If two genes cannot be distinguished by their phenotypic effect, or by any other functional criteria, they are regarded as being the same. An individual who has two identical genes is called homozygote or identical homozygote. When descendents of a common ancestor receive copies of the same gene it is said to be inbred and the consequence of inbreeding is an increased number of homozygotes at the expense of heterozygotes. As allele frequencies change, there is a chance that alleles will become fixed or lost and over a given time period, more alleles are lost in small populations. The tendency for deleterious alleles to be recessive is believed to be the genetic basis for loss of fertility and viability called inbreeding depression (Falconer & Mackey, 1996), and with higher frequency of homozygotes the probability of exposing deleterious recessive alleles increases (Frankham

et al., 2002). An example of this found in a livestock population is the complex vertebral malformation (CVM) seen in Holstein calves which results in dead, defected calves, but only if they carry two identical copies of a specific recessive allele (Agerholm, Bendixen, Andersen & Arnbjerg, 2001). The hypothesis of over-dominance states that fitness is determined by wide genomic heterozygosity level and is inherently advantageous. On one hand is the case where high fitness of the heterozygote is the consequence of variation at a single locus, called direct over-dominance and, on the other hand, the case where high fitness of the heterozygote is the consequence of variation at loci associated with a marker locus, called associated over-dominance (Hallerman, 2003). The increased frequency of either homozygote will decrease the average fitness of the population by reducing the opportunities to express over-dominance (Falconer & Mackey, 1996; Frankham *et al.*, 2002). If populations remain small and isolated for many generations, they have to deal with genetic threats, as alleles become randomly fixed or lost from the population by drift. Random genetic drift is described as the likelihood that an allele is lost by chance, causing a change in gene frequency (Lacy, 1989). Deleterious mutations will tend to accumulate, because selection is less effective in small populations. Small populations that experience inbreeding for many generations sometimes rebound in trait values despite an increasing inbreeding level. This has been referred to as purging of genetic load and can be described as a natural selection against deleterious alleles exposed by inbreeding (Aðalsteinsson, Dýrmundsson, Bjarnadóttir & Eyþórsdóttir, 1994; Keller & Waller, 2002).

Pedigree records are the most readily exploitable source of information for recognizing kinship, rate of inbreeding, mating planning and estimating other population genetic parameters (Caballero & Toro, 2000). The trend in inbreeding is one of the tools most frequently used to quantify the rate of genetic drift, by calculating the change in inbreeding per generation (ΔF) (Boichard *et al.*, 1997). The inbreeding coefficient (F) is used to measure inbreeding from pedigree information and is equivalent to the probability that both alleles at a given locus are identical by descent (IBD). If $F = 0$ there is no inbreeding and if $F = 1$ there is complete inbreeding (Frankham *et al.*, 2002). Alleles can also be identical in state (IIS), and contribute to homozygosity without inbreeding, i.e. possess identical alleles that do not descend from a common ancestor. Therefore, the degree of homozygosity can be greater than F, especially in populations or breeds that have started with only a few animals and for loci with a small number of alleles. F can be calculated through knowledge of the pedigree or estimated by determining allele frequencies after detection of genetic polymorphisms (Keller & Waller, 2002).

In order to estimate F, a base population must be established. Since the number of ancestors in a pedigree increases by 2^n per generation (where *n* is the number of generations) the pedigree increases exponentially and, eventually, all individuals are related. The base population is made of founders, animals whose parents are assumed to be unknown and treated as they are non-inbreed, that is F equals zero (Falconer & Mackey, 1996). The estimated level of inbreeding is quantified by the coefficient of inbreeding and can be defined as the probability that the pair of alleles is identical by descent (Falconer & Mackey, 1996). The standard formula for the inbreeding coefficient (Wright, 1925) is as follows:

$$F_x = \sum[(½)^n (1+F_A)]$$

where F_x is the inbreeding coefficient of the individual *x*. The number of individuals in a given path through a common ancestor is given by *n,* and the value ½ is raised to the *n*th power because in each generation, the probability is ½ that a particular ancestral allele will be transmitted from a parent to individual offspring. F_A is the inbreeding coefficient of the common ancestor A from which the lines of descent arise. Contributions to the inbreeding for each path are independent and summed to estimate the overall inbreeding coefficient for the individual in question. By calculating F, a measure of the amount of genetic diversity that has been lost can be obtained. In order to measure inbreeding on a more constant scale, ΔF can be estimated by regressing individual inbreeding coefficient on generation number. The change in inbreeding per generation can then be used to estimate the effective number of breeding animals (N_e), where:

$$N_e = \frac{1}{2 \times \Delta F}$$

1.2.2 Effective population size

Effective population size (N_e), is an estimate of the number of animals that would produce the observed rate of inbreeding in the current generation under ideal conditions (Lacy, 1995). Characteristics of an ideal population include: equal variance in family size, large number of breeders, random mating, equal sex ratio, absence of selection, mutation or migration and discrete generations (Falconer & Mackey, 1996). The effect of genetic drift under different

management strategies is dependent on the effective population size rather than the census population size (Fernández *et al.*, 2005). The absolute number of individuals in a population is a poor indicator of the population status with regard to genetic diversity. A good example of this is given by the comparison of two Portuguese breeds, the Alentejana cattle, counting 12000 females, and the Malhado de Alcobaca pigs, counting 160 females. Despite the dissimilar population size and history, the loss of genetic diversity is similar in these two populations, the average inbreeding coefficients (F) are 8.35% and 9.03%, ΔF per generation is 2.15% and 1.99%, and N_e is 23.3 and 25.1 for these two populations, respectively. The Malhado de Alcobaca pigs is a population recovering from a serious bottleneck, which explains the poor genetic status of the breed, while in the case of the Alentejana cattle the inbreeding is the consequence of the extensive use of few sires and sire families (Gama, Carolino & Vicente, 2008). In both cases the effective population size is nearly half of what is recommended as the minimum number to maintain genetic diversity in conservation, breeds with an inbreeding rate per generation > 1%, equivalent to an effective population size of < 50 individuals are considered to be in a critical state (FAO, 1998).

1.2.3 Pedigree completeness

When estimating inbreeding through pedigree analysis the pedigree completeness (PEC) is of great importance. To detect any inbreeding an animal must have at least both parents and one grandparent known, corresponding to a PEC value of 0.24 (MacCluer *et al.*, 1983; Sigurðsson & Jónmundsson, 1995). If one parent is missing then inbreeding can not be estimated and F equals zero. For animals with both parents known, its inbreeding will be underestimated if some of its ancestors are unknown. If the proportion of missing parents and ancestors is large the inbreeding trend in a population could be seriously underestimated (Lutaaya, Miszatal, Bertrand & Mabry, 1999). In particular, when mating systems are used in order to slow down the increase of inbreeding with mating of unrelated or less-related animals, lack of pedigree information can delay that process and increase both inbreeding and dominance (Lutaaya *et al.*, 1999).

Low inbreeding coefficients may arise because of a lack of pedigree information rather than the absence of inbreeding because unknown relatives are presumed to be unrelated when calculating inbreeding coefficients (Marshall *et al.*, 2002). Lutaaya *et al*. (1999) found that when the proportion of unidentified dams increased, the calculated inbreeding level decreased. For this purpose they used pedigree records from 2255 Holstein cattle with almost

complete pedigrees (less than 1% of the dams unidentified). When using regular inbreeding algorithms (RA) based on the definition of Wright they found that when 20% of dams were unidentified the level of inbreeding decreased by 60% and when 50% of dams were unidentified the inbreeding level decreased by 89%. Furthermore, they found that when using the VanRaden algorithm (VRA) the inbreeding level decreased by 30% when 20% of dams were unidentified and by 78% when 50% of dams were unidentified. Both methods show a sharp drop in calculated inbreeding when the proportion of unidentified dams increases which underlines that the reliability of pedigree analysis with incomplete pedigrees is questionable and depends heavily on the level of missing pedigree information. Boichard *et al*. (1997) analyzed the effect of missing pedigree information on the values of N_e and found that when 10% of male and 10% of female information was removed the value of N_e increased. When 20% of female information was removed, there was an even greater overestimation of N_e.

1.2.4 Genetic contribution

A founder is defined as an animal that has no known genetic relationship to animals in the pedigree other than its own descendants. Founders contain all the genetic diversity available for transmission to their descendents. Founders selected from a large population contain only a part of the genetic diversity and heterozygosity in that population, even under random selection (Lacy, 1989). A small number of founders and small family size in later generations can cause an increase in homozygosity due to inbreeding and random genetic drift (Lacy, 1989). Large founder populations and/or large family sizes tend to maintain genetic diversity and, over time, may result in gains of genetic diversity and increased heterozygosity through mutation. Mutation and migration are the only processes that can increase genetic diversity within a population (Lacy, 1989). The rate of inbreeding increases when few ancestors contribute more than others and decreases when many ancestors contribute equally. For populations undergoing mass selection, studies have shown that the rate of inbreeding is directly related to the mean and diversity of long term genetic contributions from ancestors to descendants. The rate of inbreeding is defined in terms of long-term genetic contribution by the formula:

$$\Delta F = \frac{1}{4}\sum_{i=1}^{N} r_i^2$$

where *r* is the genetic contribution of ancestor *i* and the sum is over all male and female parents (N) selected from the offspring of the base population (Woolliams & Thompson, 1994). The equation above is the simplest relationship and is not exact and has shown to underestimate the rate of inbreeding (Woolliams & Bijma, 2000).

The total number of founders gives limited information on a population genetic basis, mainly for two reasons. Firstly, founders are assumed to be unrelated which is most probably not the case. Secondly, some founders have been used more than others and have therefore contributed more to the current population (Sørensen, Sørensen & Berg, 2005). To account for unequal founder representation, Lacy (1989) estimated the effective number of founders (f_e), as:

$$f_e = \left(\sum_{i=1}^{n} p_i^2 \right)^{-1}$$

where p_i is the expected proportional genetic contribution of founder *i,* calculated by the average relationship of the founder to each animal in the current population and *n* is the total number of founders. The parameter f_e indicates the number of equally contributing founders that would produce the same level of genetic diversity as observed in the current population.

A concept similar to f_e was used by Boichard *et al.* (1997) to estimate the effective number of ancestors (f_a), defined as:

$$f_a = \left(\sum_{i=1}^{m} a_i^2 \right)^{-1}$$

where a_i is the marginal contribution of each ancestor, as opposed to each founder, to the current generation and *m* is the total number of contributing ancestors. Individual contribution to the effective number of ancestors can be used to find the most influential ancestors. It is the animal that passes its genetic material to most descendants that makes the highest contribution. Animals in the current population under study are given a value of one and marginal contributions are obtained by processing the pedigree from the youngest to the oldest. Then the ancestor with the highest contribution is chosen, their sire and dam information is removed from the pedigree, so their contribution to the population is not counted twice. The algorithm is then rerun each time an ancestor is removed and new ancestor selected (Boichard *et al.*, 1997). The effective number of ancestors is dependent on

the depth of the pedigree and is useful in comparison of the effective number of founders. The ratio of the two is an indicator of the importance of bottlenecks in the development of the population. If the effective number of founders is larger than the effective number of ancestors, bottlenecks have occurred in the population (Sørensen *et al.*, 2005). The genetic contributions of founders are independent and sum to one. That is not the case for genetic contribution of ancestors. For example, the dam of a highly used sire has at least half of the contribution of her son, because the same genetic material is represented in both generations (Boichard *et al.*, 1997).

1.2.5 Population fragmentation

When population fragmentation into subpopulations takes place, that is the separation of a population into partly or completely isolated fragments, inbreeding will develop because population size is restricted and the effect of genetic drift will increase (Keller & Waller, 2002). Small, isolated populations subjected to sustained demographic bottlenecks will rapidly lose genetic diversity through drift and the impact of population fragmentation depends on population structure and gene flow (Frankham *et al.*, 2002). Population genetics theory predicts that demographic bottlenecks will reduce both heterozygosity and allelic diversity (Nei, Maruyama & Chakraborty, 1975). Of these two measures allelic diversity appears to be the more sensitive indicator of bottleneck history (Nei *et al.*, 1975; Spencer, Neigel & Leberg, 2000). The so-called Wahlund effect refers to the reduction of heterozygosity in a population caused by subpopulation structure. If two or more subpopulations have different allele frequencies then the overall heterozygosity is reduced, even if the subpopulations themselves are in Hardy-Weinberg equilibrium. The underlying causes of this population subdivision could be geographic barriers to gene flow, followed by genetic drift in the subpopulations (Frankham *et al.*, 2002).

1.2.6 Factors influencing genetic diversity in small populations

Genetic diversity within randomly mating populations is generally increased by mutation and migration, but decreased by drift and selection. The relevant evolutionary models are either single-locus or polygenic. Marker diversity is widely used to estimate population genetic diversity for single-locus models. Studies of quantitative trait loci (QTL) indicate that physiological and morphological traits are influenced by numerous loci (Willi *et al.*, 2006).

In neutral single locus models without selection where only genetic drift and mutation occur, heterozygosity increases with effective population size mainly for two reasons. Firstly, the magnitude of genetic drift is inversely proportional to N_e and drift results in a decrease of heterozygosity at the rate of $1/(2N_e)$ per generation. Drift is rather important when a population is already declining, because each parent has a fewer offspring than expected for a population of the same constant size, increasing the chance of losing rare alleles. Secondly, fewer mutations appear in small populations. The expected number of generations until a mutation occurs is $1/(\mu N_e)$, where μ is the mutation rate per locus. In single locus models with selection, heterozygosity is assumed to decline at all population sizes, because selection increases the likelihood that the allele with the highest fitness will be fixed, although, the effect of selection is smaller when many loci are involved. Wright's definition of a locus under selection begins to behave as a neutral locus when, $s \leq 1/(2\ N_e)$, where s is the selection coefficient (Willi *et al.*, 2006). If s = 1, selection against the genotype is total, and it makes no contribution to the next generation. If s = 0, the genotype is not selected against at all. In this case gene frequencies will lapse into Hardy-Weinberg equilibrium, unless there is neutral drift (Frankham *et al.*, 2002).

For quantitative traits with polygenic inheritance under the assumption of neutrality (absence of selection) the additive genetic variance (V_A) of a quantitative trait increases linearly with N_e, as in single locus models. Polygenic models for quantitative traits with selection predict that equilibrium genetic diversity depends on factors other than population size, like the intensity of selection, the rate and effect of mutation, and the number of loci involved. As in neutral models V_A is predicted to increase with population size under all kinds of selection, because of higher mutation rate and weaker drift.

Experimental results show that heritability of quantitative traits usually declines when population size is experimentally reduced and the response to selection declines with time in small populations (Willi *et al.*, 2006). Nevertheless, not all small populations have low quantitative variation, because the outcome of natural selection becomes less predictable when the effective population size is low.

1.2.7 Inbreeding depression

Inbreeding depression has been well documented in many populations for a variety of traits, for example in cattle (Smith, Cassell & Pearson, 1998), sheep (Rzewuska, Klewiec & Martyniuk, 2005), goats (Moradi-Shaharbabak, Mohammadi & Miraei-Ashtiani, 2003) and dogs (Ólafsdóttir & Kristjánsson, 2008). Inbreeding has been shown to have a negative effect

on birth weight, offspring number, juvenile survival, longevity, mating ability, sperm quantity and quality, maternal ability, age at sexual maturity and adult survival in animals, and on related characteristics in plants (Frankham *et al.*, 2002). Moradi-Shaharbabak *et al.* (2003) found a reduction of 6.1 grams in birth weight, 24.7 grams in weaning weight and 467 grams in nine months weight for every 1% increase in inbreeding in Raeini cashmere goats. An estimate of the effect of inbreeding across several dairy cattle breeds showed that a 1% increase in inbreeding resulted in a decreased milk, fat and protein yield of 29 kg, 1.08 kg and 0.97 kg, respectively (Wiggans, VanRaden & Zuurbier, 1995). Furthermore, in highly inbred guppies (F = 0.59) sperm quality declined and the inbred males were significantly less successful in gaining paternity than their outbred rivals (Zajitschek, Lindholm, Evans & Brooks, 2009). In a wild wolf population inbreeding has been shown to affect bone development increasing the frequency of congenital malformations in the lumbosacral region of the vertebral column compared to other, less inbred, populations (Räikkönen, Vucetich, Peterson & Nelson, 2009).

1.3 Genetic variation estimated through genetic analysis

1.4.1 Mitochondrial DNA

The mitochondrion is an organelle in the cell cytoplasm, it is the only animal organelle with its own DNA and is, in most species, transmitted to offspring from mother only (Griffiths, Wessler, Lewontin & Caroll, 2008). Mitochondrion DNA (mtDNA) contains highly informative polymorphic sites and its simple maternal inheritance without recombination makes it useful for population studies in many organisms. For genetic diversity analysis the D-loop, cytochrome *b* locus and 12S rRNA locus are most commonly used. By comparing the mtDNA sequences from different individuals or species the genetic relationship can be assessed for individuals or groups within species and can also be used to identify and quantify the phylogeny (evolutionary relationship) among different species (Frankham *et al.*, 2002). Sequencing of mtDNA has advantages since mtDNA has high mutation rate and is highly variable, and it can be used to specifically trace female lines of descent, or migration patterns. Its disadvantages are that it traces only a single maternally inherited unit and mtDNA can only be considered a single 'locus' and mutations occur at different rates within the D-loop. If the founding population, before divergence, was polymorphic, then drift can lead to incorrect phylogenies (Frankham *et al.*, 2002). Mitochondrial DNA sequences have been widely used to study the origin of domestic animals like cattle (Bradley, MacHugh, Cunningham &

Loftus, 1996; Troy *et al.*, 2001), sheep (Hiendleder, Mainz, Plante & Lewalski, 2002), horse (Vilà *et al.*, 2001), dog (Savolainen, Zang, Luo, Lundeberg & Leitner, 2002), and goat (Luikart *et al.*, 2001; Sultana, Mannen & Tsuji, 2003; Naderi *et al.*, 2007). The mtDNA control region has mostly been used to describe the genetic polymorphism of goats (Luikart *et al.*, 2001; Naderi *et al.*, 2007). Luikart *et al*. (2001) assessed the phylogenetic history and population structure of domestic goats using mtDNA from 406 individuals representing 88 breeds. The sampling spanned most of the Old World from Nigeria to Iceland (six samples were collected from Icelandic goats) and Mongolia to Malaysia. The results pointed to multiple maternal origins and three mtDNA haplogroups (*Capra hircus* A-C) were observed. The main lineage (*C. hircus* A) was found in all countries including Iceland. Haplogroup B was found in Asia, Pakistan, India, Malaysia and Mongolia, haplogroup C was detected in Slovenia, Switzerland and Mongolia. Further studies have suggested the existence of three new haplogroups D, F and G. Haplogroup D was found in India (Joshi *et al.*, 2004) and haplogroups A, B, C and D were found in Chinese goat breeds (Chen, Su, Wu, Sha & Zang, 2005). Haplogroup F was found in Sicilian goats (Sardina *et al.*, 2006) and the most recently observed haplogroup G was found in Iran, Saudi Arabia, Turkey and Egypt (Naderi *et al.*, 2007).

1.4.1 Microsatellite analysis

Microsatellite DNA sequence is a type of repetitive DNA that consists of very short tandem repeats. Microsatellite marker analysis is useful for the estimation of genetic distance and relationship among closely related populations and is widely used for the study of genetic diversity in goats (Saitbekova *et al.*, 1999). Due to many favorable characteristics, such as abundance in the genome, high levels of polymorphism, co-dominance and genotyping efficiency, microsatellites are used to evaluate genetic relationship between different breeds and also to estimate genetic diversity within populations (Fan *et al.*, 2008). Molecular markers such as microsatellites are often useful when pedigree information is missing or to verify their accuracy and in studies of wild population (Frankham *et al.*, 2002). Agha *et al.* (2008) studied genetic diversity in five Egyptian and Italian goat breeds with seven microsatellite markers and found them all polymorphic; number of alleles per locus ranging from four to sixteen. The markers were highly informative in all but three of the studied breeds (PIC > 0.50). Genetic diversity within the breeds was relatively high with mean expected heterozygosity of 0.722. In twelve Chinese goat breeds the mean expected and observed heterozygosity varied from 0.611-0.784 and 0.602-0.783, respectively (Li *et al.*,

2002). In several Swiss goat breeds the average expected heterozygosity has been found to vary from 0.51-0.58 (Saitbekova *et al.*, 1999). Molecular markers are useful in detecting recent bottlenecks in a population by measurements of the number of alleles and heterozygosity at each of several loci from a population sample (Cornuet & Luikart, 1996). Genetic methods are increasingly being used to estimate effective population size (Waples & Do, 2008).

1.4 The Icelandic goat population

The Icelandic goat (*Capra hircus*) is believed to have originated from Norway and been brought to Iceland during the settlement period 874-930 (Aðalsteinsson, 1981). There is no evidence of later goat imports to the country. Records from 1703 and onwards show that goats have been kept in all parts of the country in small herds and the total number of animals in the population has been under 1000 animals in most years (Figure 1). The highest number recorded was nearly 3000 animals in 1930. The size of the population declined below 100 animals in the years 1885 and 1960 (Sveinsdóttir, 1993; Aðalsteinsson *et al.*, 1994). Around 1960, when the number of animals had fallen below one hundred, there was a growing concern that the Icelandic goat might become extinct, and since 1965 a state conservation grant has been available for recorded goats (Dýrmundsson, 1988) up to 20 animals per herd (Dýrmundsson, personal communication 2008). Compared to other Icelandic breeds: cattle, horses and sheep, which are of great economic value, much less attention has been given to the goat population, because they are mainly kept as pets. However, a few breeders keep goats for both milk and meat production.

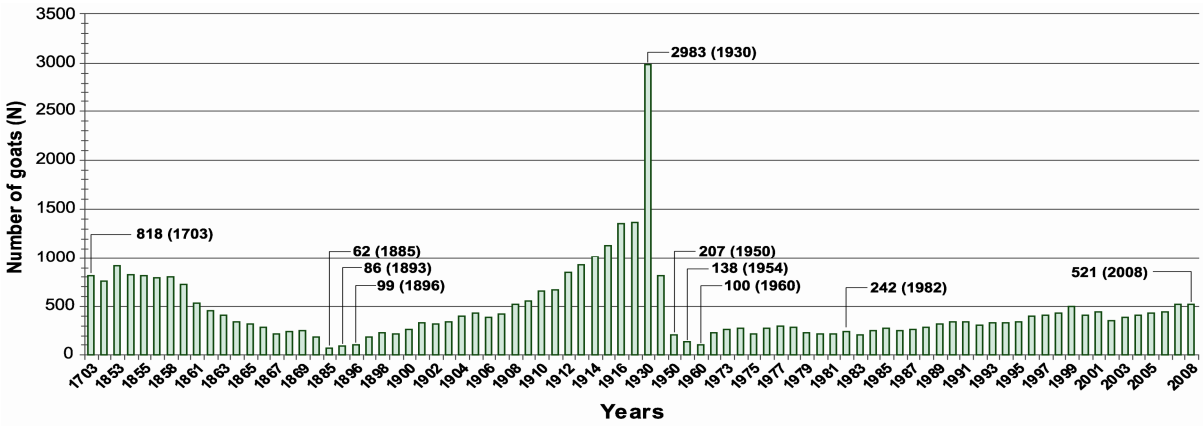

Figure 1 Population size of the Icelandic goat breed for the years 1703-2008; (Sveinsdóttir, 1993; Hagstofa, 2008) Dýrmundsson personal communication 2008).

The Goat Breeders Society of Iceland was founded in 1991 and cooperates with the Farmers Association of Iceland with the common aim of conserving the Icelandic goat and market goat derived products (Dýrmundsson, 2005). In 2007 there were 521 winterfed goats in 45 herds kept around the country (see Figure 2 for location of farms and number of goats on each farm) (Dýrmundsson, personal communication, 2008) with limited exchange of breeding animals across herds due to regulations aimed at preventing the spread of diseases (see Figure 2 for isolation zones).

In 1933, twenty sheep of the Karakul breed were imported from Germany to improve the pelt quality of Icelandic lambs. The imported sheep were carriers of diseases which were consequently introduced to the Icelandic sheep population. These included paratuberculosis, maedi-visna and Jaagsiekte (Jónmundsson & Dýrmundsson, 1988; Friðriksdóttir, Gunnarsson, Sigurðarson & Guðmundsóttir, 2000). In order to prevent the spread of these diseases the country was divided into infected and non-infected zones (Friðriksdóttir *et al.*, 2000).

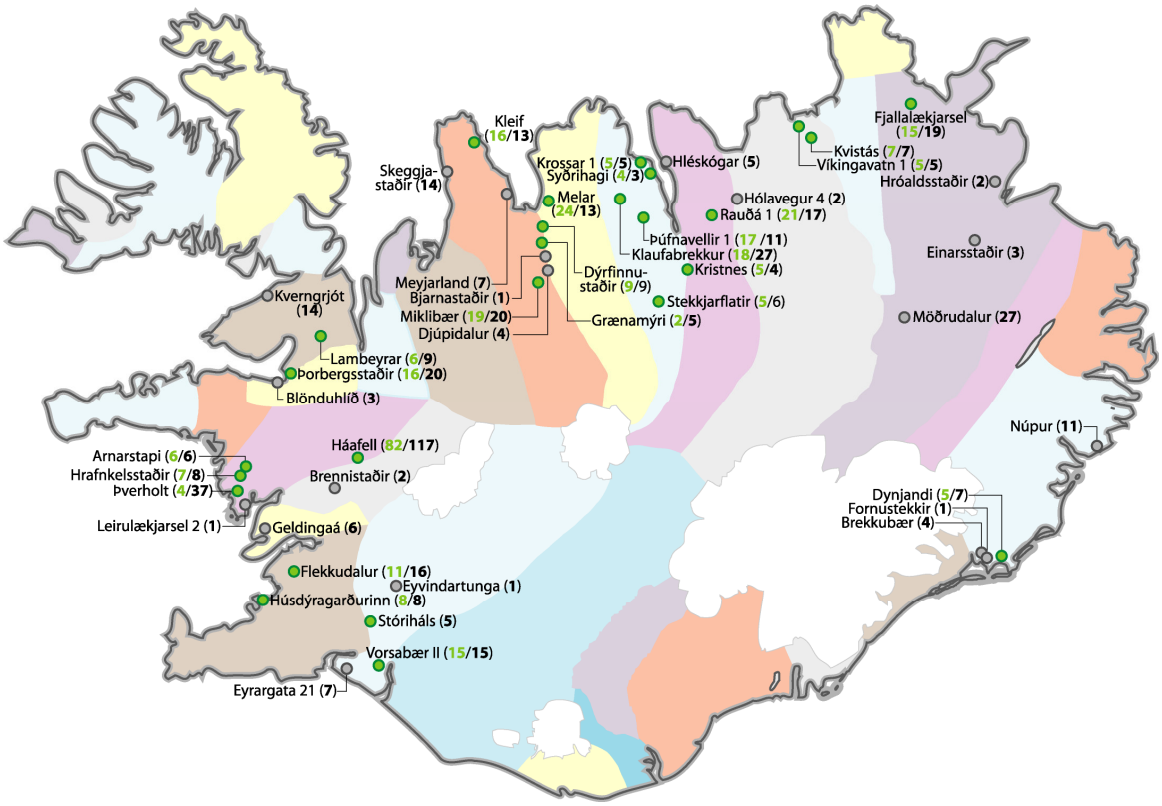

Figure 2 Location of Icelandic farms keeping goats in the year 2008. Farms where samples were collected are shown in green, others in grey. Total number of goats on each farm and number of goats sampled (shown in green) are shown in parenthesis The differently colored part indicate different regions within Iceland that have limited or no flow between them due to regulations aimed at limiting the spread of infectious diseases.

Today there are 23 zones (reduced from 36 to 23 in September 2009) (Figure 2) with limited transportation of animals (sheep, goats and cattle) between isolation zones (Matvælastofnun, 2009).

This has led to fragmentation of the goat population into very small sub-populations counting 1-30 individuals, with one exception of the Háafell Farm, where the herd counts over one hundred. The Icelandic goat population is thus a small, fragmented breeding group that has remained closed since the settlement. The breed has gone through several bottlenecks and it is therefore almost certainly heavily inbred and at a high risk of extinction.

As expected, the phenotypic variation within the population is limited. The Icelandic goat has been shown to yield fine cashmere of high quality but to produce less than most of the comparable breeds (Sveinsdóttir & Dýrmundsson, 1994). About 20% of Icelandic goats are white and 80% nonwhite with various color types, mainly piebald. Both bucks and does of the Icelandic goat breed have horns, but a few individuals are polled (Sveinsdóttir & Dýrmundsson, 1994). According to Stefán Aðalsteinsson polled sires of the Icelandic goat breed, homozygous dominant for polledness, are generally infertile with abnormal testicular development (Sveinsdóttir, 1993).

Birth weights recorded for kids are between two and three kilograms and weights for mature goats are 35-50 kg and 60-75 kg (Table 1) for females and males, respectively. Most kids are born in April-May and generally milking goats are assumed to yield about 1-2 L per day during the summer which declines to about 0.5 L in the autumn (Þorvaldsdóttir, personal communication, 2009). Sveinsdóttir (1993) found that the mean length of gestation is 149 days and 1.15 kids are born on average per doe mated. Average litter size varies between farms, the average number of kids born at Háafell Farm (98 adult does) in 2009 was around 1.45 kids per doe and 60% of does, two years and older, had two kids (Þorvaldsdóttir, personal communication 2009).

Through pedigree analysis of the Icelandic goat population with pedigree completeness index (PEC) ≥ 0,7 (128 goats and 348 matings) Aðalsteinsson *et al*. (1994) found an average inbreeding coefficient (F) of 26% in the period 1977-1992, and that a 10% increase in F, resulted in a decrease in fertility (2.8%), total number of kids born (0.8%), and kids born alive (2.6%).

Goat keeping in Iceland during the last five decades has been fluctuating and none of the farms that kept goats in 1960 keep goats today as shown in Figure 3. The main reasons for this are probably that goats have mostly been kept as pets and when diseased sheep herds

have been slaughtered, mainly Scrapie disease, uninfected goat herds within the same isolation zone have also been slaughtered.

Table 1 Comparison of the Icelandic goat population to other breeds for live weight, main use and phenotype for horns.

	Life weight		Wither height (cm)		Main use	milk yield (liters/y)	Horns (+) Polled (-)	References
	♂	♀	♂	♀				
Icelandic goat	60-75	35-50[1]			milk/meat/pet	255[2]	+/-	1) Sveinsdóttir (1993), 2) Þorvaldsdóttir (2009)
Norwegian dairy goat	80	50			milk	560	+/-	DAD-IS*
Swedish landrace goat	70	40	75	65	milk/meat		+/-	DAD-IS*
Danish landrace goat	80	58	90	80	milk/meat/pet	800	+/-	DAD-IS*
Finnish landrace goat	68	50	70	60	milk/pets		+	DAD-IS*
Irish goat	85	55	90	80	milk/wool/meat		+	DAD-IS*
Toggenburg (UK)	70	60	80	70	milk		+	DAD-IS*

*DAD-IS information retrieved from webpage 12th September 2008 at http://lprdad.fao.org/.

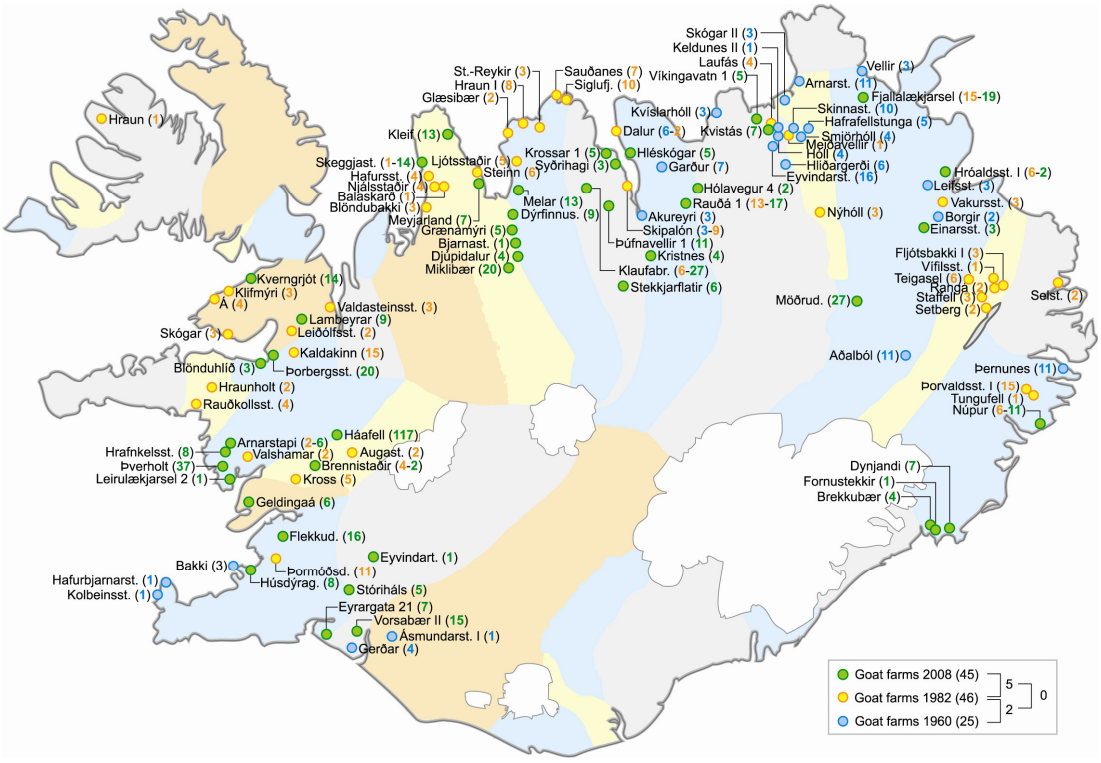

Figure 3 Location of farms keeping goats in the years 1960 (blue), 1982 (yellow) and 2008 (green).

2 Aims of study

This study has three main objectives:

Firstly, to characterize the genetic structure of the Icelandic goat population using pedigree information to calculate the level of inbreeding, effective population size, genetic contributions and related parameters important for characterizing the population structure.

Secondly, to collect DNA samples from the Icelandic goat population for analysis using D-loop sequencing as well as through microsatellite analysis to estimate the genetic structure and the genetic diversity within the goat population. In addition, the DNA collection will serve as a basis for further genetic analysis of this unique population.

Thirdly, to compare the outcome, of these two methods, in order to assess their reliability. The outcome will also serve as a basis for an organized conservation scheme for the Icelandic goat population, aimed at minimizing inbreeding and the loss of genetic diversity.

3 Material and methods

3.1 Pedigree analysis

Pedigree information in this study was obtained from the Nordic Gene Bank for Farm Animals (Nordisk Genbank Husdyr (NGH), now NordGen) and the Farmers Association of Iceland. The pedigree data included altogether 2240 animals, the oldest born in 1962 and the youngest in 2006. All animals were given an individual number including the year of birth (4 digits), sex (1 digit: 1=male, 2=female), area (2 digits) and farm number (3 digits) all together 10 digits. Inbreeding coefficient (F), pedigree completeness (PEC) and ancestors with the largest contribution for animals born in the years 2002 and 2006 were calculated with the EVA_inbred computer software (Berg, 2004). PEC values were calculated for each animal as follows:

$$PEC_{animal} = \frac{4(C_{sire} \times C_{dam})}{C_{sire} + C_{dam}}$$

where C_{sire} and C_{dam} are contributions from the paternal and maternal lines, respectively (MacCluer *et al.*, 1983). The contributions were computed as:

$$C = \left(d \times \sum_{i=1}^{d} a_i \right)^{-1}$$

where a_i is the proportion of ancestors present in generation *i, d* is the number of generations, or the depth of the pedigree. In this study, five ancestor generations were used ($d = 5$) and the PEC index therefore referred to as PEC5. Average inbreeding coefficients were calculated for the whole breed within years using the algorithm of Meuwissen and Luo (1992). The trend in inbreeding was studied for all animals and also for subclasses of animals with PEC5 ≥ 0.24, ≥ 0.50, ≥ 0.70, ≥ 0.80, the number in each group being 2240, 1059, 536, 354 and 231 animals, respectively. Generation interval (L) is the average age of parents at the birth of their offspring. The generation interval was calculated for the four pathways (father-son/daughter, mother-son/daughter) from the difference between birth dates of animals and their parents, this was done by applying the Fortran 77 software of Boichard (2002) and the mean generation interval was calculated as follows:

$$L = \frac{(L_{f\text{-}s} + L_{f\text{-}d} + L_{m\text{-}s} + L_{m\text{-}d})}{4}$$

Effective population size (N_e) was estimated from the rate of inbreeding per generation, obtained by multiplying the annual rate of inbreeding, ΔF_y, with the generation interval (L). Changes in F were obtained by regressing annual inbreeding coefficient on generation number. Following Falconer & Mackey (1996, p. 60) the rate of inbreeding is defined as:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$$

After rearrangements using the recurrence relationship the following expression is arrived at (Hartl & Clark, 1997):

$$(1\text{-}F_t) = (1\text{-}F_0)\ (1\text{-}\Delta F)^{\,t}$$

Using the logarithm the expression is transformed into a linear additive model instead of a multiplicative model:

$$\ln(1\text{-}F_t) = \ln(1\text{-}F_0) + \ln(1\text{-}\Delta F)*t = \alpha + \beta*t$$

This expression suggests that using a transformation of the empirical coefficients of inbreeding from individuals in a population, regressed against time, is a way of estimating the rate of inbreeding per generation using the estimated regression coefficient and assuming a generation interval of interval (L) (Sørensen *et al.*, 2005):

$$\Delta F = 1\text{-}\exp\,(\beta*L)$$

$$\updownarrow$$

$$N_e = \frac{1}{2(1 - \exp(\beta * L))}$$

The effective population size was estimated using the development in inbreeding of animals with PEC5 ≥ 0.80 in the years 2000-2006 (two generation intervals). Fluctuations in population size and pedigree completeness made the estimate of ΔF complicated and therefore it was necessary to limit the analysis to this time period and animals with very complete pedigrees.

Genetic relationship (R) was calculated within and between areas in order to clarify the genetic structure of the population. This was done by applying the software package Pedig (Boichard, 2002). The program used for this purpose was *Par3.f* that builds up the relationship matrix term by term by generating progeny for each parent pair of interest and computes the inbreeding coefficient with Meuwissen`s method (Meuwissen & Luo, 1992). The relationship between the parents of interest is then two times the inbreeding coefficient of the artificial offspring.

3.2 Sample collection and DNA extraction

DNA samples were collected from Icelandic goats in the period of July 2007 to April 2008. A total of 350 samples, both blood (83) and tissue (267), were collected from a total of 26 farms out of the total of 45 goat farms in Iceland (Figure 2). Genomic DNA and mtDNA from blood samples was extracted from buffy coat using the MasterPure™ DNA purification Kit (EPICENTRE® Biotechnologies). Tissue samples were collected from buccal (cheek) using BuccalAmp™ DNA swabs and extracted with QuickExtract™ DNA Extraction Solution (EPICENTRE® Biotechnologies) according to manufacturer's recommendations. Samples for analysis were selected from the sample collection such that samples were analyzed from all farms. For storage DNA samples were diluted to a concentration of 100 ng/μL and stored at -20°C.

3.3 PCR amplification and DNA sequencing

The goat mtDNA is 16,640 bp in length and the D-loop is 1212 bp in length (position 15,429-16,640) (Parma, Feligini, Greppi & Enne, 2003). A 598 base pair segment of the mtDNA D-loop was sequenced, spanning positions 15,652 – 16,251. The primers ChirDL-F2 (5´-CGT GTA TGC AAG TAC ATT AC-3´) and ChirDL-R1 (5'GAT GGA CTA ATG ACT AAT CAG-3') were used to amplify the mtDNA fragment. For PCR amplification a working dilution of 10 ng/μL of genomic DNA was used. A 25μL PCR reaction was performed using

12.5 µL of Taq 2x Master Mix (as supplied by New England BioLabs®) which included 0.4 mM dNTPs, 50 U/ml Taq polymerase, 3.0 mM $MgCl_2$, Standard Taq Reaction Buffer and stabilizers, 1µL of each primer and 2 µL $MgCl_2$.

The PCR amplification was done on a Px2 Thermal Cycler (Thermo Electron Corporation) using the following setup: an initial denaturation at 96°C (4 min), annealing at 53°C (45 sec), and extension at 72°C (1.5 min) for 35 cycles. After amplification the PCR products were run on a 1.5% agarose gel stained with ethidum bromide and visualized by UV light exposure. Bands of the correct size were excised from the gel and purified using NucleoSpin® Extract II PCR clean-up Gel extraction kit (Macherey-Nagel GmbH & Co. KG) according to the manufacturer's recommendations and the DNA eluted in 40 µL of elution buffer. The purified PCR product was checked by running it on a 1.5 % agarose gel. For sequencing, the appropriate primers were added to the samples and then they were sequenced at MWG-Biotech AG (www.eurofinsdna.com).

3.4 Mitochondrial DNA analysis

Samples that yielded useful sequence data were aligned, manually inspected, and edited using Geneious Pro (Drummond *et al.*, 2009). A total of 49 sequences were obtained from the Icelandic goat population. In order to compare the sequences from this analysis to previously published data, sequences were obtained from GenBank (www.ncbi.nlm.nih.gov). A total of 141 previously published sequences from 15 North European countries were used for comparison: From Austria (AT) [EF617678-80, EF617685], Denmark (DK) [AJ317650, EF617710], England (UK) [AJ317592, AJ317841, EF617729], France (FR) [AJ317575, AJ317713, AJ317723-25, AJ317629-30], Germany (DE) [AJ317586, AJ317627-28, AJ317649, EF617788-93, EF617800-801], Iceland (IS) [AJ317587, EF617851-55], Ireland (IR) [AJ317588-91, EF618085-86], Norway (NO) [AJ317593-95], Poland (PL) [AJ317584-85, AJ317651-52, EF618264, EF618280], Slovakia (SK) [AJ317653-54], Slovenia (SI) [AJ317731, AJ317835, AJ317837, EF618346-50], Sweden (SE) [AJ317637, EF618415-22], Switzerland (CH) [AJ317573-74, AJ317596-99, AJ317605, AJ317619-24, AJ317626, AJ317631-36, AJ317836, AJ317838, AJ317638-48, EF618423-26], Ukraine (UA) [AJ317600-604, EF618540], Wales (WA) [AJ317655-58, EF618542-44] and Capra ibex [AJ317871] (Luikart *et al.*, 2001; Naderi *et al.*, 2007). Representing the six domestic goat haplogroups (A, B, C, D, F and G) the following sequences were used for haplogroup A: [AY155721] (Joshi *et al.*, 2004), [EF617779, EF617945, EF617965, EF618134 and

EF618200] (Naderi *et al.*, 2007), for haplogroup B1: [AB044303] (Mannen, Nagata & Tsuji, 2001) and [EF617706] (Naderi *et al.*, 2007), for haplogroup B2: [AJ317833] (Luikart *et al.*, 2001) and [DQ121578] (Liu, Lei & Yang, 2006) for haplogroup C: [AJ317838] (Luikart *et al.*, 2001), [AY155708] (Joshi *et al.*, 2004), [DQ188892] (Liu *et al.*, 2006) and [EF618413] (Naderi *et al.*, 2007) for haplogroup D: [AY155952] (Joshi *et al.*, 2004), [DQ188893] (Liu *et al.*, 2006) and [EF617701] (Naderi *et al.*, 2007) for haplogroup F: [DQ241349 and DQ241351] (Sardina *et al.*, 2006) and for haplogroup G: [EF617727, EF618084 and EF618535] (Naderi *et al.*, 2007). Sequences were analyzed using the software program Geneious Pro (Drummond *et al.*, 2009). Haplotypes were examined and phylogenetic tree was constructed with Tamura-Nei neighbour-joining methods.

3.5 Genetic diversity analysis

Fifteen microsatellite markers were used for analysis: CSRD0247, ILSTS008, ILSTS019, ILSTS087, INRA023, INRA172, INRA063, MAF065, McM0527, OarFCB11, OarFCB20, SRCRSP23, SRCRSP05, SRCRSP08, and INRA06. The markers are distributed over the 29 of caprine autosomal chromosomes. All of the markers are jointly recommended by the FAO and the International Society for Animal Genetics (ISAG) for analysis of genetic diversity of goats (Hoffmann *et al.*, 2004). Samples were genotyped at MWG-Biotech AG (www.eurofinsdna.com).

Several estimators were used to analyze the marker data, including the basic diversity indicates; total numbers of observed alleles (N_{OA}), allele frequency, mean number of alleles (MNA), observed (H_O) and expected (H_E) heterozygosity and HWE. The Hardy-Weinberg equilibrium (HWE) implies that allele frequencies are constant from one generation to the next. If a population deviates from HWE some evolutionary force (e.g. selection, mutation, migration and drift) is changing the allele frequencies between generations. The HWE for each locus were calculated with GenAlex software (Peakall & Smouse, 2006). The HWE over the whole population was estimated using GENEPOP program version 4 (Rousset, 2008). The polymorphism information content (PIC) was calculated for each marker, which refers to the ability of a given marker to detect polymorphism within a population, depending on the numbers of detectable alleles and their frequency (Guyomarc´h, Sourdille, Charmet, Edwards & Bernard, 2002). The higher the PIC value is, the more informative the marker and a locus with PIC value > 0.5 is regarded as highly informative whereas a locus with a PIC <

0.25 is regarded as slightly informative (Botstein, White, Skolnick & Davis, 1980). For this purpose POWERMARKER software (Liu & Muse, 2005) was used.

Two methods were used to estimate whether the Icelandic goat population had experienced a recent genetic bottleneck. The first method is based on the fact that for natural loci, allele number and frequency distribution result from equilibrium between mutation and genetic drift. The parameters of this mutation-drift equilibrium are the mutation rate and N_e (Cornuet & Luikart, 1996). If the heterozygosity observed from the samples of genes is significantly greater than the heterozygosity expected from the number of alleles found in the sample under mutation-drift equilibrium, then the population has exhibited heterozygosity excess which indicates a recent genetic bottleneck. Two different statistical test were applied, a sign test and a Wilcoxon test. These tests were used on three different models of microsatellite evolution. Briefly, the infinite allele model (IAM) is based on the equilibrium between the loss of diversity caused by drift and the introduction of a new mutations, each mutation produces a new allele that is different from the existing ones; the stepwise mutation model (SMM) accounts for the exact changes of an allele caused by mutation before reaching steady state (Cornuet & Luikart, 1996) and the two-phased model of mutation (TPM) represents an intermediated stage between the other two, incorporating the mutation process of the SMM while allowing for mutations of a larger magnitude to occur (Murray, 1996). The second approach involves a graphical method that groups alleles into ten frequency classes and then plots a frequency histogram. The graphical method concludes that a population has been recently bottlenecked if fewer alleles are found in lower frequency classes (0.001-0.100) than in one or more intermediate frequency classes (0.101-0.900) (Luikart, Allendorf, Cornuet & Sherwin, 1998). Both approaches were carried out using the computer software program BOTTLENECK (Piry, Luikart, Cornuet & 1999) (www1.montpellier.inra.fr/URLB/ bottleneck/bottleneck.html) performing 1000 replicates (Cornuet & Luikart, 1996). The BOTTLENECK software program is designed to identify recently bottlenecked populations, "recently" being defined by the authors of the program as a few dozen generations (Luikart & Cornuet, 1998; Piry *et al.*, 1999).

For estimation of N_e within the Icelandic goat population using microsatellite data LDNe 1.31 (www.fish.washington.edu/xfer/LDNE) (Waples & Do, 2008)) was applied to estimate N_e based on linkage disequilibrium.

4 Results

4.1 Pedigree analysis

4.1.1 Pedigree completeness and trend in inbreeding

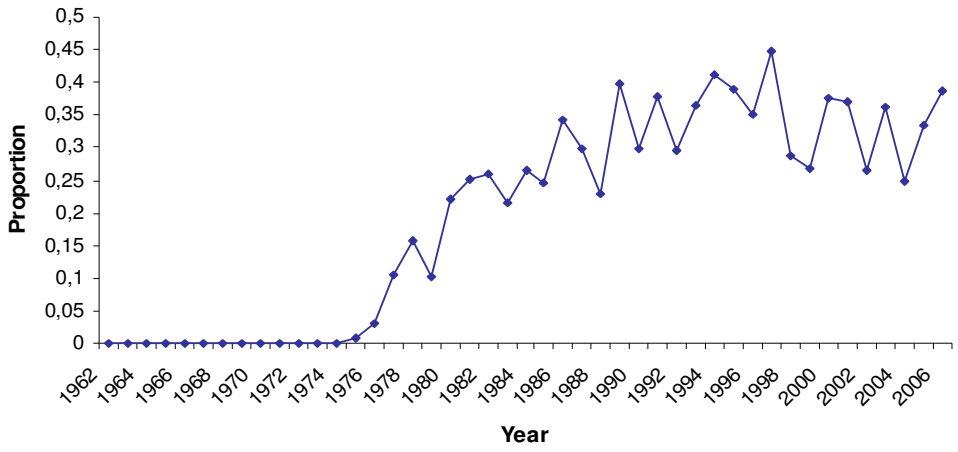

Figure 4 Proportion of available pedigree data for the Icelandic goat population**.**

The proportion of available pedigree records in 2006, shown in Figure 4, was 38.7 % with the highest proportion in 1997 (47.3%). Only eight animals in the data set had PEC5 = 1.

The mean inbreeding coefficient was calculated within years for all animals and animals with different PEC5 indices. In 2006 the number of goats recorded for PEC5 ≥ 0.24 ≥ 0.50; ≥ 0.70 and ≥ 0.80 were 38, 24, 10 and 6, respectively. Inbreeding was first detected in 1974 where the proportion of inbred animals calculated was 2% where as in 2006 the proportion had increased heavily and 62.5% of animals were inbred (Figure 7). The mean coefficient of inbreeding for goats born in 2006 for all animals and animals representing each PEC5 subclass ≥ 0.24, ≥ 0.50, ≥ 0.70, ≥ 0.80 was 10.5%,15.9%, 19.3%, 31.5% and 50.4% respectively (Figure 5). Inbreeding was first detected in 1974 for all animals (0.5%) and animals with PEC5 ≥ 0.24 (25%), in 1978 for animals with PEC5 ≥ 0.50 (18.8%), in 1981 for animals with PEC5 ≥ 0.70 (21.5%) and in 1984 for PEC5 ≥ 0.80 (45.8%). The highest inbreeding coefficient was calculated in 1985 with PEC5 ≥ 0.80 (64.4%).

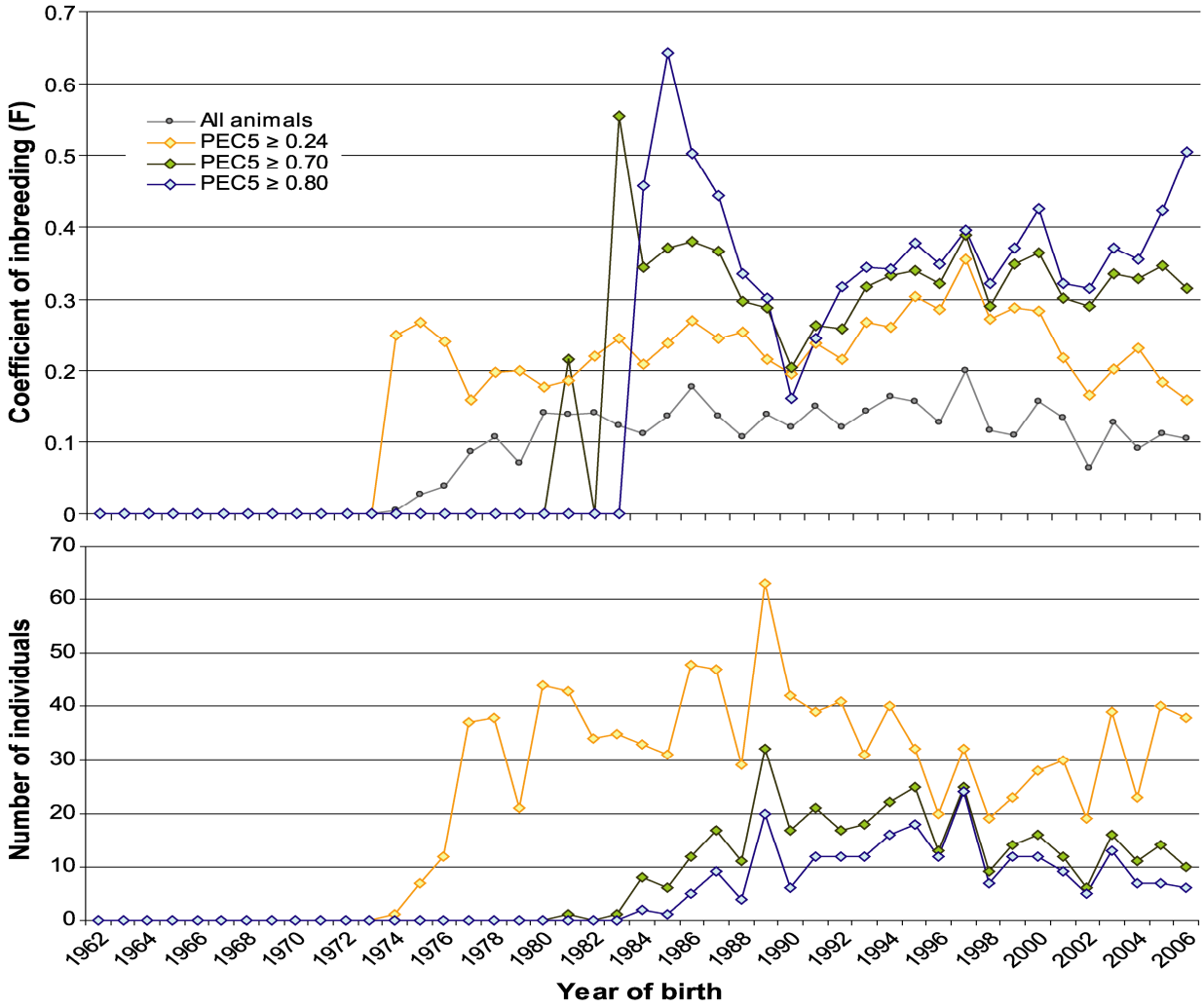

Figure 5 Development in inbreeding (F) during the years 1962-2006 (above). Number of animals representing each PEC5 subclass index (below).

The ten most inbred goats in the Icelandic goat population in the period 1962-2006 computed for the whole dataset are shown in Table 2. The five most inbred animals were all from the same farm Á located in Dalasýsla. Hatta and Fífa born in 1986 and 1987 full-sib female goats from the farm Á with PEC5 index of 0.93 were 71.1% inbred. Sproti and Höttur (males) born in 1986 and 1985 and Ögn (female) born in 1984 were all full sibs 64.4% inbred and Ögn is the mother of Hatta and Fífa (see pedigree chart, Figure 6). They came from a sub-population that started with individuals 1976238001, 1978138001 and 1973238001 that also were inbred. The females 1976238001 and 1973238001 were half-sibs and the male 1978138001 was also related to the two female goats. All of the ten most inbred goats had PEC5 ≥ 0.87.

Table 2 The ten most inbred goats found within the Icelandic goat population in the period 1962-2006, based on pedigree data.

Name of animal and farm (ID number)	Birth year	Inbreeding	PEC5 index
Hatta from Á (1986238060)	1986	71.1%	0.93
Fífa from Á (1987238060)	1987	71.1%	0.93
Sproti from Á (1986138060)	1986	64.4%	0.87
Höttur from Á (1985138060	1985	64.4%	0.87
Ögn from Á (1984238060)	1984	64.4%	0.87
Huðna from Rauðá (1999266104)	1999	59.3%	1
Fönn from Stóri Háls 2006287101)	2006	58.7%	0.96
Blíða from Núpur (1989276060)	1989	57.6%	0.96
Lýsa from Steinn (1991257101)	1991	56.7%	0.87
Brá from Fjallalækjarsel (2005267001)	2005	56.1%	0.99

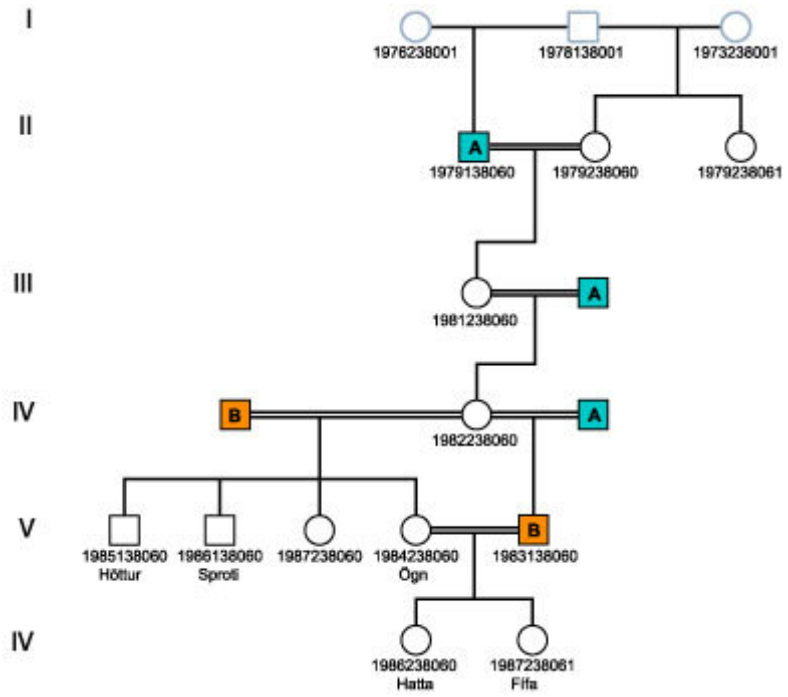

Figure 6 A pedigree chart spanning six generations for the Á Farm where the five most inbred goats were found shown in Table 2.

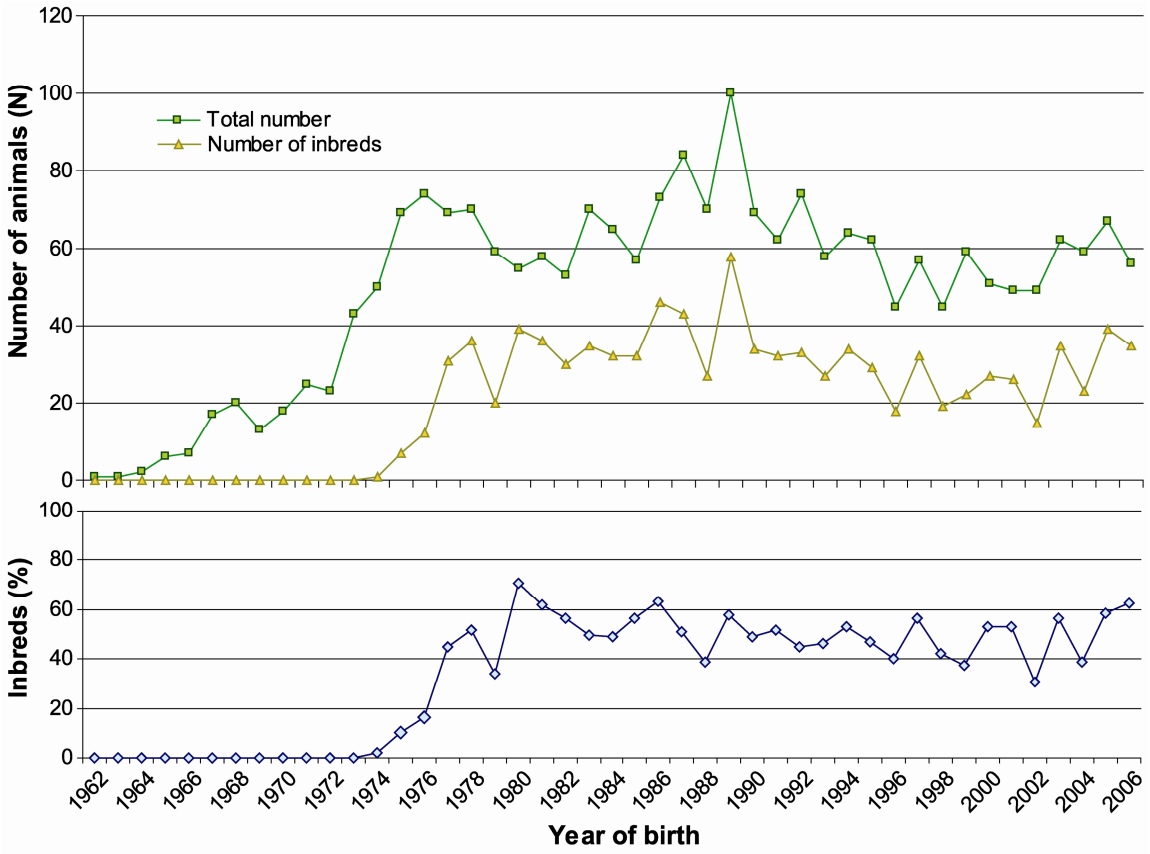

Figure 7 Total number of animals (above) in the Icelandic goat population (green line), total number of inbred animals (yellow line), and proportion of inbred animals (below).

The highest proportion of inbred animals was in the 1980 year-class (70.9%). The highest individual genetic contribution to the Icelandic goat population (shown in Table 3) was calculated for the years 2002 and 2006. The doe Veiga from Sólheimar, contributed the most in 2002 (9.5%) and the highest contribution in 2006 comes from the buck, Glanni from Háafell (16.5%). Glanni's high contribution in 2006 is the consequence of his extensive use in 2005 and 2006, as he is the sire of 17% of the kids born in 2005 and 19.2% of the kids in 2006.

Table 3 Animals with the largest genetic contribution in the years 2002 and 2006. Number of individuals representing each year was 45 animals.

Year 2002		Year 2006	
Name of ancestor and farm (ID number)		**Name of ancestor and farm (ID number)**	
Veiga from Sólheimar (1994287120)	9.5%	Glanni from Háafell (2004136001)	16.5%
Þorri from Sólheimar (1990187120)	7.8%	Veiga from Sólheimar (1994287120)	9.5%
Dagur from Fjallalækjarsel (1984167002)	7.7%	Þorri from Sólheimar (1990187120)	9.0%
Heimir from Sólheimar (1999187120)	7.7%	Baugalín from Háafell (2003236001)	8.3%
Dreki from Fjallalækjarsel (1978167001)	7.2%	Heimir from Sólheimar (1999187120)	7.5%
Rjómalind from Fjallalækjarsel (1973267001)	6.3%	Hlunkur from Háafell (2001136001)	6.6%
Höttur from Fjallalækjarsel (1980167001)	6.2%	Slembi from Vorsabær (1987187080)	6.2%
Rjúpa from Fjallalækjarsel (1964267001)	6.1%	Keisara from Vorsabær (1978287080)	6.0%
Bogi from Fjallalækjarsel (1983167001)	5.8%	Örn from Þorbergsstaðir (2003138100)	5.4%
Prins from Háafell (2000136001)	5.1%	Hnokki from Sólheimar (1994187120)	4.9%

4.1.2 Effective population size

Generation interval for father-son, father-daughter, mother-son and mother-daughter was 2.9 years, 3.5 years, 3.1 years and 4.5 years, respectively. Mean generation interval for all animals born in 1962-2006 was L = 3.5 years. The increment in inbreeding over one generation, estimated using animals with PEC5 $\geq$ 0.80 born in the years 2000-2006, was found to be 9.9%. Based on these values the effective population size of the Icelandic goat populations was estimated to be N_e = 5.1 animals.

4.1.3 Relationship within and between areas

Relationship coefficient (R) was calculated within and between areas shown in Figure 8 and Appendix 1 for the year intervals 1990-1999 and 2000-2006. Relationship within areas increased for all areas from 1990-2006 except for Eyjafjörður (65) and Norður-Þingeyjarsýsla (67) where relationship declined from 10.4% to 2.5% and 21.2% to 13.8%, respectively (Figure 8, red squares). Highest increase in relationship within areas was in Reykjavik (01) and Kjósarsýsla (16) where relationship increased from 5.3% to 39.7% and 5.1% to 32.7%, respectively. Relationship within areas was higher than between areas in all cases except for areas 01 and 67 (90-99) and areas 38 and 87 (00-06).

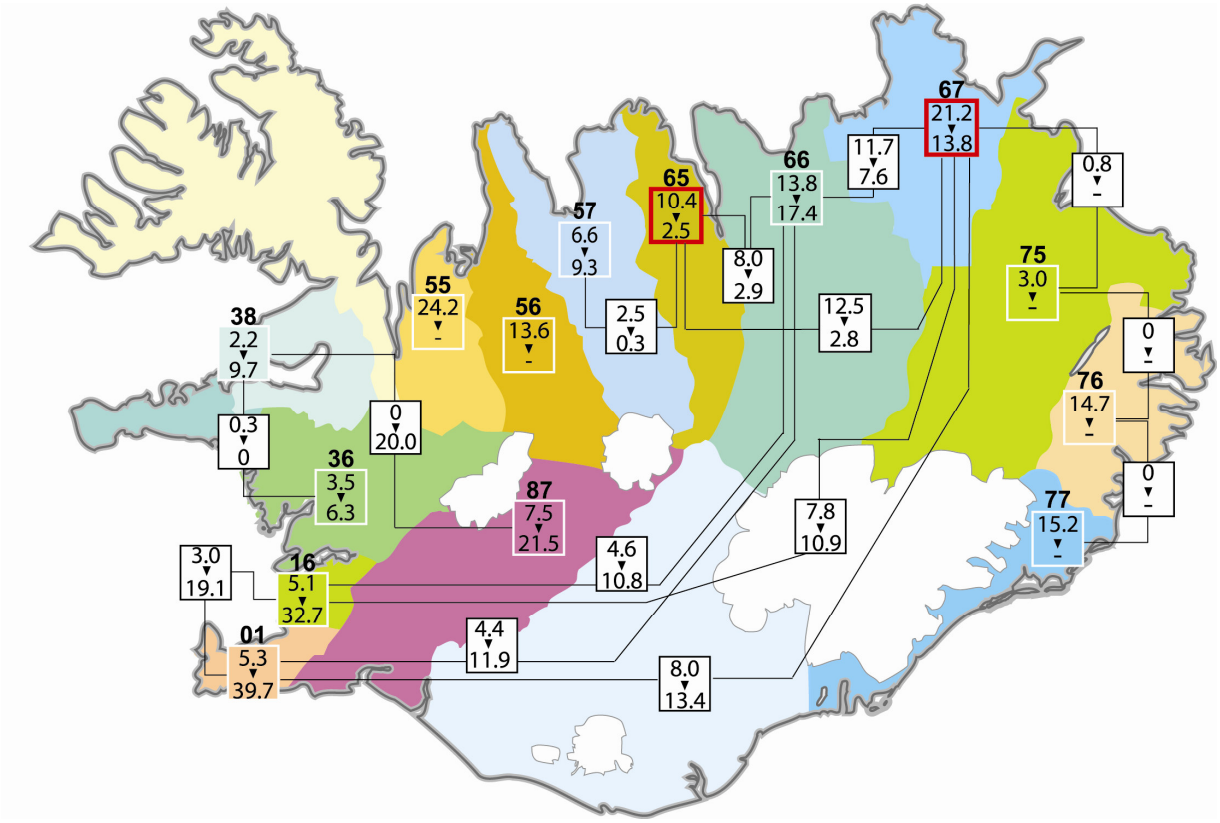

Figure 8 Relationship within and between areas. Colored squares show the within area relationships and white squares show relationship between areas. Within each square are shown relationships from two time periods that are 1990-1999 (above) and 2000-2006 (below). Above each square is the area code and the red squares show the two areas where the relationship within has declined between the two time intervals. The areas are numbered as follows: Reykjavik (01), Kjósarsýsla (16), Borgarfjörður (36), Dalasýsla (38), Vestur-Húnavatnssýsla (55), Austur-Húnavatnssýsla (56), Skagafjörður (57), Eyjafjörður (65), Suður-Þingeyjarsýsla (66), Norður-Þingeyjarsýsla (67), Norður-Múlasýsla (75), Suður-Múlasýsla (76), Austur-Skaftafellssýsla (77) and Árnessýsla (87).

4.2 Microsatellites diversity

A total of 52 samples were analyzed, revealing 27 alleles across the 15 loci giving a mean number of 1.8 alleles per locus (MNA). Six loci were monomorphic, seven loci had two alleles, one had three alleles and one had four alleles as shown in Table 4. For two loci with two alleles CSRD0247 and McM0527 the frequency of the most common allele exceeded 0.950 so they can be considered monomorphic (Hartl & Clark, 1997). Taking this into consideration, eight of the fifteen (53%) loci can be considered as monomorphic. The frequency of the most common allele for marker INRA06 is high (0.912) but does not exceed 0.950 and thus has to be regarded as polymorphic. Genetic diversity measures showed mean observed heterozygosity (H_O) of 0.178, or 0.364 when excluding monomorphic markers, and

mean expected heterozygosity (H_E) of 0.185 (SE ± 0.054), or 0.307 when excluding monomorphic markers. The expected frequencies are computed according to the Hardy-Weinberg equilibrium, using the values of allele frequencies observed. Two loci (ILSTS087 and SRCRSO23) showed significant ($P< 0.001$) deviation from HWE according to the exact test. Significant deviations from HWE were found for the same markers when using chi-square test ($P< 0.001$). When examined over all loci the result showed significant deviation from HWE ($p < 0.001$). The inbreeding coefficient (F_{IS}) was estimated over all loci as 2.6% (see Table 4 for values for each locus).

Table 4 Diversity indices calculated for fifteen microsatellite markers. Number of samples (N), chromosome number (Chr), number of observed alleles (N_{OA}) and reported values, size range in base pairs, reported values for other goat breeds included, frequency of alleles heterozygosity (observed (H_O), expected (H_E)), within population inbreeding estimates (F_{IS}) and polymorphism information content (PIC). The allele with the highest frequency for each marker is given in italics. The highest and lowest values for H_O and H_E are underlined.

		Number of observed alleles (N_{OA})		Size range of alleles						
Marker	**N (Chr)**	**Ice**	**Rep**	**Iceland**	**Reported**	**Allele frequency**	**H_O**	**H_E**	**F_{IS}**	**PIC**
CSRD0247	49 (14)	2	9	238; *240*	221–247[2]	0.041; 0.959	0.082	0.078	-0.043	0.075
ILSTS08	51	1	8	172	166–184[1]	1.00	0.000	0.000	na	0.000
ILSTS019	51 (25)	1	8	148	145–159[1]	1.00	0.000	0.000	na	0.000
ILSTS087	50 (6)	3	11	133; 134; *143*	136–158[1]	0.430; 0.020; 0.550	<u>0.540</u>	<u>0.512</u>	0.054	0.427
INRA023	50 (1)	1	10	195	198–218[3]	1.00	0.000	0.000	na	0.000
INRA172	51 (26)	2	6	145; *149*	238–250[4]	0.402; 0.598	0.490	0.481	0.020	0.365
INRA063	51 (18)	2	7	*171*; 173	171–181[2]	*0.794*; 0.206	0.294	0.327	0.101	0.274
MAF065	52 (15)	2	12	117; *135*	112–136[3]	0.154; *0.846*	0.192	0.260	0.261	0.226
McM0527	50 (5)	2	7	164; *168*	152–168[2]	0.020; *0.980*	<u>0.040</u>	<u>0.039</u>	0.020	0.038
OarFCB11	52 (2)	1	11	142	120–160[5]	1.00	0.000	0.000	na	0.000
OarFCB20	51 (2)	1	8	107	93–117[2]	1.00	0.000	0.000	na	0.000
SRCRSP23	51	4	15	*81*; 83; 95; 97	85–123[2]	*0.598*; 0.029; 0.363; 0.010	0.471	0.510	0.077	0.415
SRCRSP05	50 (21)	1	13	173	158–182[3]	1.00	0.000	0.000	na	0.000
SRCRSP08	49	2	10	*244*; 260	211–240[3]	*0.724*; 0.276	0.388	0.399	0.029	0.320
INRA06	51 (3)	2	13	*121*; 123	100–130[5]	*0.912*; 0.088	0.176	0.161	0.097	0.148
Mean		**1.8**	**9.9**				**0.178**	**0.185**	**0.026**	**0.153**

1) (Fatima, Bhong, Rank & Joshi, 2008), 2) (Di Stasio, 2009), 3) (Li *et al.*, 2002), 4) (Luikart *et al.*, 1999), 5) (Menezes, Martinez, Ribeiro, Filho & Bermejo, 2006)

Based on the marker analysis N_e for the Icelandic goat population is estimated to be 4.1-8.8 individuals (lower and upper 95% confidence limits set as 2.2 and 21.6, respectively).

The mode-shift test showed that most alleles were in the lowest frequency classes, indicating no recent bottleneck, although the data did not show the typical 'L' shaped distribution characteristic for non-bottlenecked populations shown in Figure 9.

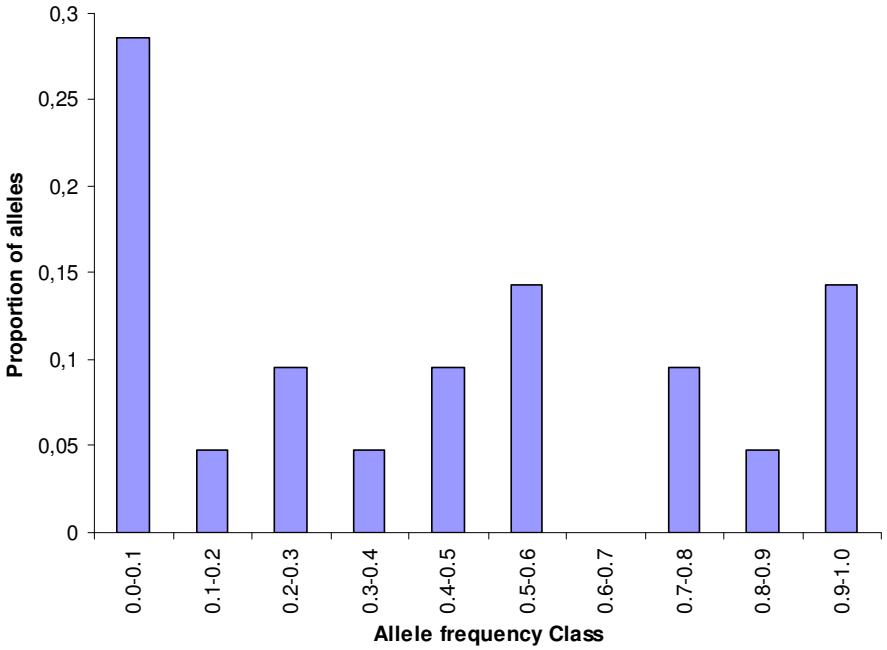

Figure 9 A mode-shift curve showing distribution of alleles in different allelic classes.

Both the sign test and Wilcoxon rank test were in all cases insignificant indicating no recent bottleneck.

4.3 Mitochondrial analysis

Diversity in polymorphic sites was examined by comparing sequences from this study with the reference sequence from NCBI databank [NC005044] covering the complete sequence of the goat mitochondrial genome. The control region sequences showed twelve variable sites and three haplotypes were observed in the D-loop samples investigated, shown in Table 5. Two of the haplotypes (represented by ChIce 030 and 050) varied only in one polymorphic site in nucleotide position number 15871.

Table 5 Three mtDNA haplotypes found in Icelandic goats, nucleotide position number indicate the positions of polymorphic sites.

	15809	15840	15871	15885	15911	15928	15971	15972	15981	16019	16026	16158
NC_005044	T	A	A	G	G	T	A	T	G	C	G	C
ChIce_197	C	G	A	C	A	C	A	C	G	T	A	A
ChIce_030	C	A	G	C	G	C	G	T	A	C	A	A
ChIce_050	C	A	A	C	G	C	G	T	A	C	A	A

A neighbour-joining tree was constructed and for this purpose data from previous studies of known mitochondrial haplogroups (A, B, C, D, F and G), one wild goat *Capra ibex*, and data from North European goat breeds and from this study. All the sequences from the Icelandic goats and most of the North-European goats represent the most common goat mtDNA haplogroup A shown in Figure 10.

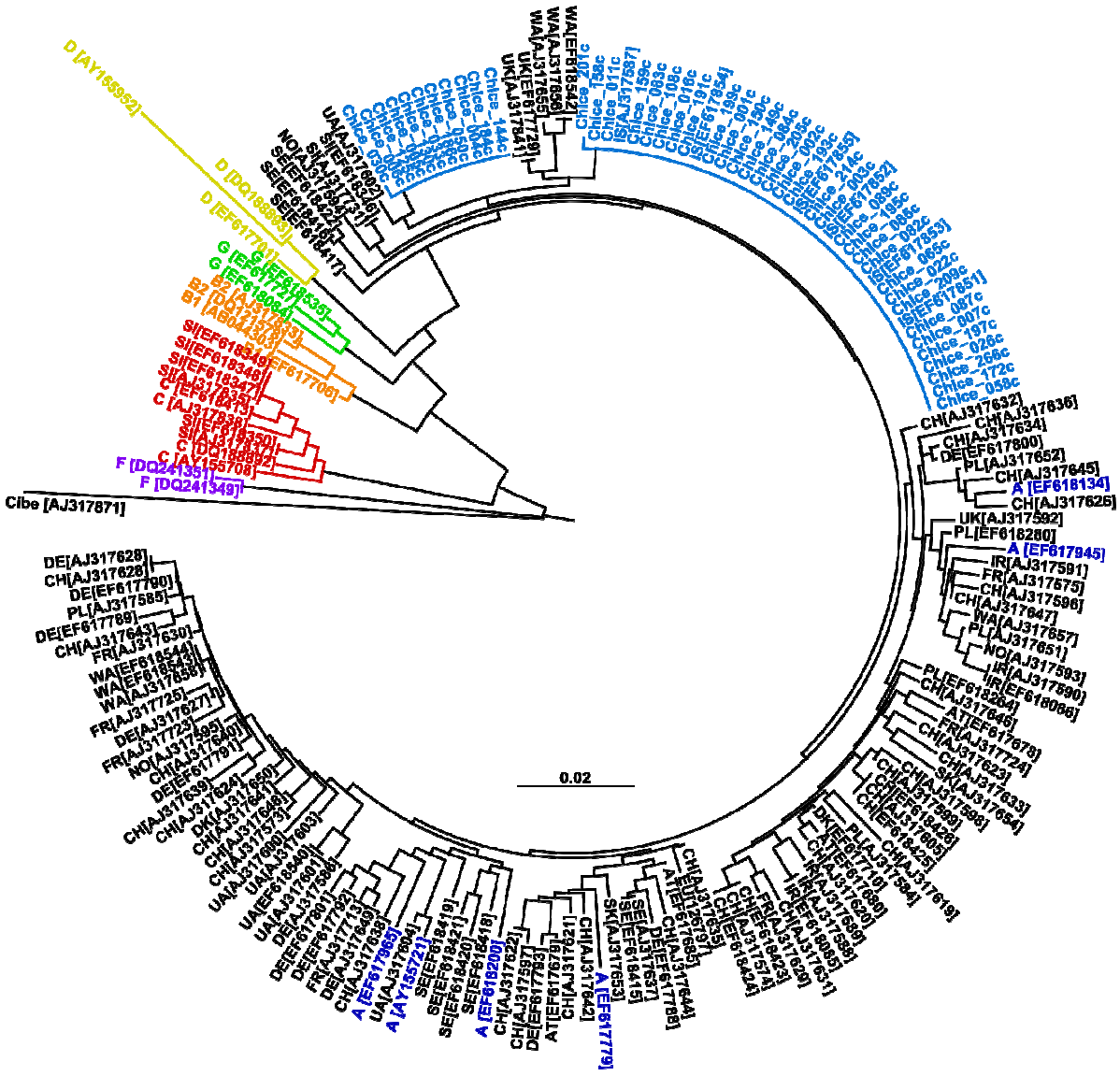

Figure 10 Neighbour-joining tree showing six known goat mtDNA haplogroups (A, B, C, D, F and G, shown in colours), mtDNA sequences from North European goat breeds, in abbreviation for each country and accession numbers shown in parentheses. Sequences from this study and previously reported sequences from Icelandic goats (shown in light blue).

5 Discussion

5.1 Pedigree analysis

Studies have shown that the completeness of pedigree information has an effect on the estimates of inbreeding coefficients within a breed (Sigurðsson & Jónmundsson, 1995; Lutaaya *et al.*, 1999). A large proportion of missing parents in a pedigree may cause serious underestimation of the inbreeding level and overestimation of the effective population size (Boichard *et al.*, 1997; Lutaaya *et al.*, 1999). The proportion of available pedigree records (PEC5) for the Icelandic goat population is quite low. In 2006 it was 38.7% and has been fluctuating between years with the highest proportion of 44.7% in 1997 (Figure 4). The proportion of available pedigree data for other Icelandic breeds with PEC5, for the cattle breed it was 56% in 2006 (Kristjánsson, Jónmundsson & Benjamínsson, 2006) and for the horse breed it was 72% in 2001 (Kristjánsson, 2003). In the year 2006 the proportion of animals with PEC5 $\geq$ 0.24 was 47.3% and PEC5 $\geq$ 0.70 was 15.8% and only eight animals in the whole data set had 100% pedigree information for five generations. This suggests that the results presented here may seriously underestimate the inbreeding level in the population. It is essential to register the pedigree data for the Icelandic goat population with more accuracy in the future in order to be able to monitor the rate of inbreeding in a more precise way and steer the conservation effort in the right direction. The fact that goats are mainly kept as pets and are not subjected to formal breeding program, which could improve their genetic productive ability, is probably the main reason for the poor pedigree recording.

In the Icelandic goat population the present inbreeding coefficients are high. In 2006 the mean inbreeding coefficient of animals with PEC5 $\geq$ 0.24 is 15.9% and for animals with PEC5 $\geq$ 0.70 is 31.5% and the proportion of inbred animals was 62.5%. The inbreeding coefficient for animals with PEC5 $\geq$ 0.70 is higher than found by Aðalsteinsson *et al.* (1994) for the period 1977-1992. The animals with the highest inbreeding coefficients had acceptable ancestor information (PEC5 $\geq$ 0.87, shown in Table 2) which may lead to the conclusion that more accurate pedigree data might reveal even higher inbreeding levels. This is seen in the tendency of increased inbreeding associated with more complete pedigree information shown in Figure 5. As pointed out above, the inbreeding coefficients are very sensitive to the quality of available pedigree information, and thus the absolute inbreeding coefficient levels provide less information for comparative purpose than the average rate of increase in inbreeding per generation (ΔF). The increase in inbreeding per generation (ΔF)

was estimated to be 9.9% and is extremely high compared to results for other goat breeds, such as for Italian Girgentana goats 0.13% (Portolano, Finocchiaro, Todaro, Kaam & Giaccone, 2004), Dutch Landrace goats 0.19% (Mucha & Windig, 2009) and for Saanen and LaMancha goat breeds 0.25% and 0.15%, respectively (Gipson, 2002). According to the FAO guidelines and the recommendation of Bijma (2000), a rate of inbreeding of more than 1% per generation should be avoided to maintain fitness in a breed (FAO, 1998; Bijma, 2000). The rate of inbreeding in a closed population is proportional to the genetic drift and thus the loss of genetic diversity (Sørensen *et al.*, 2005). This shows that it is necessary to control the future rate of inbreeding to avoid further loss of genetic diversity.

Generation interval for the Icelandic goat breed was longer than found for Dutch Landrace goats, 1.9 years (Mucha & Windig, 2009) and Italian Girgentana goat breed, 2.5 years (Portolano *et al.*, 2004). One possible explanation for the longer generation interval may be inbreeding depression, as inbreeding is known to affect all aspects of reproduction (e.g. age at sexual maturity, sperm production, mating ability) (Frankham, 2005). It can also be explained by the fact that goats are mainly kept as pets.

It has been recommended, as a rule of thumb, that the N_e of small populations should be larger than 50 individuals to prevent inbreeding depression from becoming a serious problem (FAO, 1998; Franklin & Frankham, 1998) and that N_e should be 500-5000 to retain genetic diversity and thereby the long term evolutionary potential of the population. However, it has also been recommended to maintain a N_e of at least 50 to 100 to take into account mutation and drift (Meuwissen, 1999; Bijma, 2000). Meuwissen (1999) also argued that a N_e below 100 animals leads to a decrease in population fitness. Sørensen *et al.* (2005) pointed out that the recommendations are by no means magic numbers, but have been derived from theoretical arguments, where natural selection counteracts inbreeding depression. The N_e estimate of 5.1 animals for the Icelandic goat population is far below these recommendations, which indicates that the population is facing serious genetic problems and is at high risk of extinction in the near future. Loss of genetic diversity in small populations is expected to increase extinction risk by adversely affecting the ability to cope with environmental changes such as in climate, pollution and in diseases (Frankham, 2005). In the literature reviewed the lowest N_e value found based on pedigree data was for the Japanese Black cattle ($N_e = 14$) (Nomura, Honda & Mukai, 2001), Alentejana cattle ($N_e = 23.3$) and Malhado de Alcobaca pigs ($N_e = 25.1$) (Gama *et al.*, 2008). Furthermore, for Danish Red and Danish Holstein cattle the N_e has been estimated as 47 and 49, respectively (Sørensen *et al.*, 2005).

High genetic contributions of few ancestors leads to increased inbreeding (Woolliams & Thompson, 1994). The ancestors with the highest genetic contribution in the years investigated, 2002 and 2006, contributed 9.5% and 16.5%, respectively, as shown in Table 3. The extremely high contribution of the buck Glanni from Háafell Farm in 2006 can be explained by his extensive use on the Háafell Farm where the herd numbers over one hundred goats, which represents nearly a quarter of the Icelandic goat population.

Relationship within areas was in most cases higher than between areas which indicate that gene flow between areas is limited indeed. However, taking into consideration the level of missing pedigree data the relationship could be greater between areas. Regulations that limit transportation of animals between infected and non-infected isolation zones in order to prevent the spread of diseases in Iceland are the main reason for the fragmentation of the population into small sub-populations. Only a few farms have been allowed to supply goats for breeding between zones. Exemptions from these regulations have caused controversy between goat and sheep farmers in the past. This has limited the gene flow between fragments and increased the relatedness within fragments. It is therefore necessary to break up the isolation of the fragments so that genetic material can be shared and the population can become one breeding group. This requires concessions of the regulations that limit transportation of goats between zones, increased effort from the breeders as well as dedication from advisors that can advise on breeding strategies that minimize the rate of inbreeding, e.g. by a more widespread use of sires. It has been pointed out that the sire breeding part of a population largely governs the rate of inbreeding (Goddard & Smith, 1990; Rochambeau, Fournet-Hanocq & Khang, 2000) and it has been found out from simulation studies, for example, that breeding schemes that use more sires result in lower rate of inbreeding (Korpiaho, Stranden & Mäntysaari, 2002). One way of doing this is to put the emphasis on semen collection around the country and AI, which so far has not been used to any real extent. This would also open up the possibility of semen storage as a backup for genetic material for future generations which would give breeders even more choices in their breeding work. Helpful tools like mating programs (Sonesson & Meuwissen, 2001; Berg, 2004) that choose the best parents to the next generation aiming at minimizing the rate of inbreeding and increasing the genetic diversity can be applied. The computer program EVA (Berg, 2004) was used on data from the Icelandic horse population and the results showed that there was a great possibility of reducing the rate of inbreeding in the population, partly by using more sires (Kristjánsson, 2007).

5.2 Diversity analysis

5.2.1 Microsatellite

Genetic diversity measures revealed a poor status of biodiversity in the Icelandic goat breed. The mean number of alleles was 1.8 with numbers varying from one to four alleles per locus, this suggests that many alleles have been lost and is similar to results seen in *Capra ibex* populations where MNA varied from 2 to 2.8, (Maudet *et al.*, 2002) and Sorraia horse breed in Portugal which has a MNA = 3.3 (Luis, Cothran & MarOom, 2007), but considerably lower than found for other goat breeds such as the Indian Gohiliwari, MNA = 10.12, (Kumar *et al.*, 2009); Swiss goat breeds, MNA = 7.25 (Saitbekova *et al.*, 1999), Egyptian Baladi goats, MNA = 7.6, (Agha *et al.*, 2008)). Genetic diversity observed in the population is low (H_E = 0.307 for the 9 polymorphic loci). It is lower than values reported in other ungulate species for example *Capra ibex*, $H_E \approx 0.40$, (Maudet *et al.*, 2002) ; caribou reindeer, $H_E \approx$ 0.46, (Wilson, Stronbeck, Wu & Coffin, 1997); American wapiti, $H_E \approx 0.45$ (Polziehn, Hamr, Mallory & Strobeck, 2000)), Sorraia horse breed, H_E = 0.459 (Luis *et al.*, 2007). Moreover, heterozygosity was extremely low (H_E = 0.185) if all 15 loci were considered, including the several monomorphic loci. This genetic diversity is among the lowest reported from analysis of microsatellites in mammals, including a *Capra ibex* study that found H_E = 0.13 (Maudet *et al.*, 2002) and a study on a Kodiak Island brown beer population that found H_E = 0.27 (Peatkau, Waits & Clarkson, 1998).

The Icelandic livestock breeds; goats, cattle, horses and the sheepdog, were brought to Iceland during the settlement around 900 AD, and are believed to have remained closed populations since then. Molecular estimates have shown that the level of heterozygosity in the Icelandic sheepdog population is relatively high (H_E varying from 0.60 to 0.84 for individual locus) and MNA = 11.7, despite that the population underwent a drastic bottleneck in the last century and the current population descends from only a few individuals. However, the inbreeding coefficient is rather high (0.21) (Ólafsdóttir & Kristjánsson, 2008). Furthermore, molecular diversity analysis for the Icelandic cattle population has also shown that there exists a considerable level of heterozygosity in the population (H_E = 0.685 and MNA = 6.2) (Ásbjarnardóttir, 2008).

The statistical assessment of the informativeness of a marker, denoted by PIC values, varied between 0.15 (INRA006) and 0.43 (ILSTS087) for polymorphic markers with mean PIC of 0.31, which is regarded slightly informative (< 0.5). Reported PIC values for these markers in other goat breeds have shown that they are well suited for genetic diversity

analysis in goats (Agha *et al.*, 2008; Fan *et al.*, 2008; Fatima *et al.*, 2008). However, it is difficult to compare among studies because some of these studies have only tested a few loci (< 20), different marker sets have been used and some may not have reported monomorphic loci.

This study revealed that the Icelandic goat population was not in Hardy-Weinberg equilibrium, as could be expected considering the breeds history.

Identifying populations that have experienced a severe reduction of size is important because bottlenecks can increase demographic stochasticity, inbreeding, loss of genetic diversity and fixation of deleterious alleles and thereby increase the probability of population extinction (Frankham, 2005). The Icelandic goat population is known to have experienced at least two serious bottlenecks, in 1885 and 1960, when the population was reduced to 62 and 100 animals, respectively. The mode shift test shown in Figure 9 did not reveal a recent bottleneck in the population. However, it can be seen on the histogram that alleles in the intermediate frequency classes show high frequencies, and the histogram does not have the typical 'L' shaped distribution (Luikart *et al.*, 1998) characteristic for non-bottlenecked populations as seen for the Icelandic cattle breed (Ásbjarnardóttir, 2008). In a population at mutation-drift equilibrium (e.g. effective size that has remained constant in the recent past) there is approximately an equal probability that a locus shows genetic diversity excess or deficit (Luikart & Cornuet, 1998). The methods based on heterozygous excess do not reveal a recent bottleneck even though the population has suffered known bottlenecks in the past. Substantial substructure probably exists in the population due to the fragmentation which might obscure the heterozygosity excess expected in a bottlenecked population (Cornuet & Luikart, 1996). Studies of *Capra ibex* with known bottlenecks gave similar results, but when the population was separated into two geographical sub-populations the results gave a significant bottleneck signature (Maudet *et al.*, 2002).

The N_e values based on microsatellite markers are consistent with the values estimated from pedigree data in this study. The effective population size is similar to the one found for Chillingham cattle ($N_e \approx 8$) which is an extensively studied breed that is considered genetically uniform but has remained viable and fertile despite at least 300 years of total inbreeding (Visscher, Smith, Hall & Williams, 2001).

The within inbreeding coefficient for the Icelandic goat population (mean $F_{IS} = 2.6\%$) estimated from the microsatellite data was much lower than the ones estimated using pedigree data in this study. Simulations have shown that a link between heterozygosity and inbreeding is most likely in "extreme" breeding systems such as might occur in small closely related

populations, but the correlation of heterozygosity and inbreeding is weak or undetectable with a moderate number of markers (Balloux, Amos & Coulson, 2004). Furthermore, a theoretical and empirical data both suggest that the correlation between multilocus heterozygosity and inbreeding coefficient (F) is weak, unless the studied population exhibits a relatively large variance in F (Slate *et al.*, 2004).

From these results it can be seen that a number of alleles have become fixed and others lost by drift. Considering the viability of the population and the extremely low genetic diversity it can be concluded that deleterious alleles have been purged, as has been proposed that when combined with selection, inbreeding may purge deleterious alleles (Aðalsteinsson *et al.*, 1994; Keller & Waller, 2002).

Microsatellite markers combined with recent statistic methods represent useful tools for the conservation and management in populations and should be combined with other classical (e.g. demographic) approaches. Furthermore, achieving reasonable assignment accuracy generally requires molecular data from a large number of markers (Maudet *et al.*, 2002). However, many factors might interact with the success in using these methods when populations have reached a threshold in genetic diversity.

5.2.2 Mitochondrial analysis

Analysis of mitochondrial DNA sequences from the Icelandic goat population identified only three haplotypes, all belonging to the most common goat haplogroup A. Two of the observed haplotypes only differed in one polymorphic site. Genetic diversity of goat mtDNA haplogroups has been studied on a large scale (Luikart *et al.*, 2001; Naderi *et al.*, 2007) and have shown high diversity and six haplogroups. Naderi *et al.* (2007) reported 1540 haplotypes among 2430 individuals, Sultana *et al*. (2003) identified 38 haplotypes among 44 individuals of Pakistani goats and Sardina *et al*. (2006) observed 33 haplotypes among 67 individuals of Sicilian goat breeds demonstrating the high diversity among goats.

A neighbour-joining tree of 55 new and published mtDNA sequences from the Icelandic goat population, sequences from other North European countries and sequences representing the six known haplogroup show that there is a consistent clustering pattern of the Icelandic samples into two groups (the two main haplotypes). This implies that there are two maternal lines existing in the population. On the branch closest to the Icelandic goats were goats from Wales, England and Ukraine, shown in Figure 10.

6 Conclusions

The findings presented here are in accordance with the known history of the Icelandic goat breed, namely that it is a small, heavily inbred, closed population and the status of genetic diversity is extremely poor.

Currently the pedigree data for the Icelandic goat population is poor and not sufficient to monitor the breed's status. This problem might have been solved using molecular methods, which enable the reconstitution of pedigrees, but that would have required a certain level of genetic diversity within the population. The extreme lack of genetic diversity in the Icelandic goat population measured by both microsatellite analysis and D-loop sequencing makes it very important to put an increased emphasis on collecting more pedigree data as that is the most cost effective way to monitor the breed's status as regards inbreeding.

In addition to increased efforts in monitoring the population with regard to genetic diversity other methods are also plausible. For example, derogations in the regulations that limit transportation of goats between isolation zones could increase the flow of genetic material between sub-populations and slow down the deterioration of the population. Also, semen collection and AI could be used to break up the isolation of the sub-populations. Mating programs should also be applied so as to select the best parents in order to minimize the rate of inbreeding in the population.

In the light of ever decreasing global genetic diversity, where for example approximately one goat breed becomes extinct each year, it is of great importance to protect the Icelandic goat breed from further genetic erosion and then turn the tide so as to secure a sustainable future for this unique breed, which has remained closed for 1100 years.

Further studies to evaluate the genetic diversity of the Icelandic goat breed are necessary as well as studies aimed at the increased application of semen collection, long term semen storage and artificial insemination. This work should be done in the context of a larger long term conservation plan based on a detailed population viability analysis. This should then be coupled to increased utilization of the goat breed and with strong emphasis on product development.

7 References

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Appendix 1

Relationship coefficients (R%±SD) within and between areas in the years 1990-1999 and 2000-2006. Number of animals in each area are shown in parenthesis The areas are numbered as follows: Reykjavik (01), Kjósarsýsla (16), Borgarfjörður (36), Dalasýsla (38), Vestur-Húnavatnssýsla (55), Austur-Húnavatnssýsla (56), Skagafjörður (57), Eyjafjörður (65), Suður-Þingeyjarsýsla (66), Norður-Þingeyjarsýsla (67), Norður-Múlasýsla (75), Suður-Múlasýsla (76), Austur-Skaftafellssýsla (77), and Árnessýsla (87).

		01	16	36	38	55	56	57	65	66	67	75	76	77	87
01	'90-'99 (15) '00-'06 (13)	5.3±12.0 39.7±10.4													
16	'90-'99 (10) '00-'06 (12)	3.0±7.3 19.1±4.7	5.1±10.3 32.7±8.2												
36	'90-'99 (91) '00-'06 (147)	0 0.9±1.3	0 0.7±1.1	3.5±7.7 6.3±7.2											
38	'90-'99 (35) '00-'06 (18)	0 0	0 0	0.3±1.8 0	2.2±6.4 9.7±7.9										
55	'90-'99 (9) '00-'06 (0)	0 -	0 -	0 -	0 -	24.2±13.9 -									
56	'90-'99 (6) '00-'06 (0)	0 -	0 -	0 -	0 -	0 -	13.6±18.6 -								
57	'90-'99 (93) '00-'06 (31)	1.5±5.4 1.4±2.9	1.4±4.5 1.1±2.3	0 6.1±0.2	0 0	0.9±1.5 -	0 -	6.6±11.6 9.3±11.4							
65	'90-'99 (52) '00-'06 (56)	5.0±8.9 4.7±4.7	4.8±7.0 4.0±3.8	0 0.2±0.6	0 0	0 -	0 -	2.5±6.0 0.3±0.9	10.4±10.8 2.5±5.4						
66	'90-'99 (99) '00-'06 (16)	4.4±8.1 11.9±9.3	4.6±6.8 10.8±7.9	0 0.5±1.0	0 0	0 -	0 -	2.3±5.6 0.8±2.1	8.0±8.3 2.9±3.9	13.8±14.9 17.4±18.7					
67	'90-'99 (34) '00-'06 (48)	8.0±13.6 13.4±13.6	7.8±10.6 10.9±10.7	0 0.6±1.4	0 0	0 -	0 -	4.1±9.3 1.1±3.6	12.7±11.6 2.8±4.5	11.7±11.3 7.6±10.6	21.2±17.9 13.8±20.0				
75	'90-'99 (20) '00-'06 (0)	0 -	0.3±1.1 -	0 -	0 -	0 -	0 -	0.2±1.0 -	0.5±1.6 -	0.5±1.5 -	0.8±2.3 -	3.0± 8.0 -			
76	'90-'99 (4) '00-'06 (0)	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	14.7±2.2 -		
77	'90-'99 (11) '00-'06 (0)	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	15.2±11.5 -	
87	'90-'99 (120) '00-'06 (39)	3.0±4.4 5.3±2.2	1.3±2.6 4.2±1.7	0.1±0.9 3.2±5.1	0 20.0±1.3	0 -	0 -	0.8±2.4 0.4±0.8	2.4±3.6 1.2±1.3	2.2±3.4 3.0±2.6	3.8±5.3 3.6±4.0	0.2±0.6 -	0 -	0 -	7.5±12.1 21.5±13.0

Appendix 2 Sample origin and individual mtDNA sequence number.

Name of farm	**Sequence number**
Háafell	001, 002, 003, 004, 005, 006, 007, 011, 016, 022, 026, 030, 033, 040, 050, 058, 065, 082
Fjallalækjarsel	083, 084, 085, 086, 087, 089
Kleif	108
Ljótsstaðir	125, 130, 144
Flekkudalur	149, 150, 158
Vorsabær	159, 172
Dynjandi	184, 186
Lambeyrar	191, 193
Arnarstapi	195, 197, 199
Þorbergsstaðir	201, 205, 209, 214
Rauðá	262, 266, 272
Hrafnkelsstaðir	282
Þúfnavellir	301