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Diversity of the Icelandic goat breed assessed using population data

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Abstract
The Icelandic goat breed is a closed population consisting of around 700 animals kept in 45 herds. Several population bottlenecks are known to have occurred and the population has at least twice declined below 100 animals. Here the genetic diversity of the breed was estimated using pedigree information, D-loop sequencing and microsatellite markers. The annual rate of inbreeding was estimated as 3% and the effective population size as 5.1 animals. The effective population size based on molecular markers was estimated in the range of 4.1–8.8 individuals and mtDNA D-loop sequencing identified only three haplotypes. The results presented here show the population to be highly inbred, fragmented and the level of genetic variation among the lowest found in livestock. The results will be an important input into conservation planning for the Icelandic goat.

Keywords: *Genetic diversity, pedigree analysis, microsatellites, D-loop, closed population, inbreeding depression, bottlenecks.*

Introduction

Both wild and domesticated species face great environmental changes over time and genetic diversity is required for populations to adapt to these changes. The primary goals in the management of animal populations are, therefore, to maintain the level of genetic diversity high and the level of inbreeding low (Fernandez et al., 2005). For this to be possible and to estimate the future breeding potential of a given breed it is necessary to characterize its genetic structure and to estimate the within population genetic diversity.

During the last 20 years, hundreds of animal breeds identified by the Food and Agriculture Organization (FAO) have become extinct including numerous goat breeds (Taberlet et al., 2008). This occurs despite the fact that goats are a widespread livestock species of great economic importance in many developing countries with an increasing importance in western countries (Luikart et al., 2001). Fortunately market demands are changing in some parts of the world with a growing demand for specialty products, giving breeders of rare breeds an opportunity to expand their stock and conserve genetic diversity.

The Icelandic goat (*Capra hircus*) is believed to have been brought to Iceland from Norway during the settlement period around 1100 years ago (Adalsteinsson, 1981). There is no evidence of later goat import to the country and records from 1703 and onwards, as well as archaeological remains, show that goats were kept in most parts of the country (Adalsteinsson et al., 1994). The population is known to have gone through several bottlenecks and has at least twice declined to under 100 animals, in the years 1885 and 1962. The population size has been less than 1000 animals most of the time, with the highest number, nearly 3000 animals, recorded in 1930 (Adalsteinsson et al., 1994). Around 1960, the number of goats in Iceland fell below 100, which led to a growing concern that the population might become extinct, and since 1965 a conservation subsidy has been available for goats up to 20 animals per herd. In the last decades goats have mostly been kept without production aims, with the exception of a few breeders that keep goats for milk and meat production. Little is known about the structure of

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the population or levels of genetic diversity although the level of inbreeding and its effect on the fitness of the breed has been estimated, showing only an insignificant effect of increases in inbreeding on fertility, litter size at birth and number of kids born alive (Adalsteinsson et al., 1994). In addition to the previously mentioned fluctuations in population size, the country has been divided into isolation zones, aimed at controlling the spread of sheep diseases, since the middle of the twentieth century. This limits the movement of sheep, cattle and goats between zones and may have led to the fragmentation of the goat population into sub-populations with little exchange of genetic material between zones.

The aim of the current study was to characterize the genetic structure of the Icelandic goat population using both pedigree data and analysis of molecular markers. The resulting information, such as the level of inbreeding, effective population size and coefficient of relationship, will be a valuable input into conservation planning for the breed and will serve as basis for a conservation scheme aimed at minimizing inbreeding and loss of genetic diversity.

Material and methods

Pedigree data and sample collection

Pedigree information 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 a total of 2240 animals, the oldest born in 1962 and the youngest born in 2006. All animals were given a composite ID number consisting of the year of birth (first four digits), sex (one digit, 1 = male, 2 = female), area (two digits) and a three digits unique identifier.

Blood samples were collected from goats on six farms. Sample Ch012 from Arnarstapi (64° 46,184′N, 23° 37,313′W), sample Ch033 from Fjallalækjarsel (66° 8,821′N, 15° 43,687′W), samples Ch002, Ch007, Ch013, Ch014, Ch015, Ch016, Ch018, Ch021, Ch027, Ch028, Ch029, Ch030, Ch031, Ch032, Ch034, Ch035, Ch042, Ch044, Ch046, Ch047, Ch048, Ch049, Ch050 from Háafell (64° 42,557′N, 21° 15,100′W), samples Ch003, Ch004, Ch005, Ch008, Ch009, Ch010, Ch011, Ch019, Ch020, Ch022, Ch036, Ch037, Ch039, Ch040 Ch023, Ch024 and Ch025 from Sólheimar (64° 3,936′N, 20° 38,558′W), samples Ch001, Ch006 and Ch017 from Þorbergsstaðir (65° 3,798′N, 21° 46,086′W) and samples Ch026, Ch038, Ch041, Ch043 and Ch045 from Vorsabær (64° 2,398′N, 20° 32,555′W). DNA was isolated from buffy coat by using MasterPure DNA isolation kit from Epicentre according to the manufacturer's instructions (www.epicenter.com).

Pedigree completeness

Pedigree completeness (PEC) is of importance when estimating inbreeding through pedigree analysis. To detect 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). PEC values were calculated for each animal as:

$$\mathrm{PEC}_{\mathrm{animal}} = \frac{2(C_{\mathrm{sire}} \times C_{\mathrm{dam}})}{C_{\mathrm{sire}} + C_{\mathrm{dam}}}$$

By using the EVA inbred program, where C_{sire} and C_{dam} are contributions from the paternal and maternal lines, respectively (Sørensen et al., 2008). The contributions were computed as:

$$C = \frac{1}{d}\sum_{i=1}^{d} a_i$$

where a_i is the proportion of ancestors present in generation i, and 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. The average PEC5 index was calculated according to the year of birth. Average inbreeding coefficient for animals with PEC5 values of 0.24, 0.50, 0.70 and 0.80 were calculated to ascertain the extent to which the completeness of the pedigree affects the results.

Inbreeding coefficient

The EVA inbred program (Sørensen et al., 2008) was used to calculate individual inbreeding coefficients and average inbreeding coefficients within birth cohorts 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 $\geq$ 0.24, $\geq$0.50, $\geq$0.70 and $\geq$ 0.80, the numbers in each group being 2240, 1059, 536, 354 and 231 animals, respectively.

Generation length

The generation length (L), that is the average age of parents at the birth of their offspring, was calculated for the four gametic pathways: buck to son ($L_{\mathrm{f-s}}$), buck to daughter ($L_{\mathrm{f-d}}$), doe to son ($L_{\mathrm{m-s}}$) and doe to daughter ($L_{\mathrm{m-d}}$) from the difference between birth dates of animals and their parents using the Pedig

software package (Boichard, 2002). The average generation length was calculated as:

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

Effective population size

The effective population size (N_e) is an estimate of the number of breeding animals that would produce the observed rate of inbreeding in the current generation under ideal conditions (Lacy, 1995). The effective population size (N_e) was estimated from the rate of inbreeding per generation (ΔF), obtained by multiplying the annual rate of inbreeding (ΔF_y), with the generation length (L).

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

Changes in F were obtained by regressing annual inbreeding coefficients on generation number as (Falconer & Mackay, 1996):

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

The effective population size was estimated by 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 it necessary to limit the analysis to this time period and animals with more complete pedigrees.

Ancestors with the highest contribution

It has been shown that the rate of inbreeding is directly related to the long-term genetic contributions from ancestors to descendants and large genetic contributions of few ancestors' leads to increased inbreeding (Woolliams & Thompson, 1994). The ancestors that contributed the most to individuals born in the years 2002 and 2006 were found using EVA inbred program (Sørensen et al., 2008).

Coefficients of relationship

The country is divided into isolation zones with limited exchange of breeding animals between zones. The regions were numbered as shown in Table I. The coefficient of relationship (R) was calculated within and between regions for two time periods 1990–1999 and 2000–2006 in order to clarify the genetic structure of the population and the development of this relationship using the software package *Pedig* (Boichard, 2002). The relationship matrix was built up term by term by generating progeny for each parent pair of interest and the inbreeding coefficient computed according to Meuwissen and Luo (1992). The relationship between the parents of interest is then two times the inbreeding coefficient of the artificial offspring.

Phenotypic analysis

Coat colour variation within the Icelandic goat breed was assessed using individual records submitted by farmers. This was done at three time points at 10 year intervals, the winters of 1988–1989, 1998–1999 and 2008–2009. The number of animals with coat colour information was 75, 160 and 399 for the three periods, respectively. The coat colour patterns were grouped into seven categories, solid white or tan, solid gray, badger-face, and four groups of piebald; black, gray, tan and red. Also analysed were the numbers of polled goats in the three intervals.

Microsatellite analysis

Fifteen microsatellite markers were used for analysis (see Table II), eleven of which are jointly recommended by 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). Variables used to analyze the data included number of observed alleles (N_{OA}), major allele frequency (MAF), mean number of alleles (MNA), observed heterozygosity (H_O), and expected heterozygosity (H_E). The Hardy-Weinberg equilibrium (HWE) for each locus was calculated with GenAlex 6.4 (Peakall & Smouse, 2006). The HWE for the population was estimated using GENEPOP version 4 (Rousset, 2008). POWERMARKER (Liu & Muse, 2005) was used to calculate the polymorphism information content (PIC) for each marker, a measure of the ability of a given marker to detect polymorphism within a population dependent on the number of detectable alleles and their frequency (Botstein et al., 1980).

Two methods were used to test for recent genetic bottlenecks. First a method of heterozygosity excess, where an excess in observed heterozygosity over expected heterozygosity is considered an indicator of a recent genetic bottleneck. Two statistical tests were applied, a sign test and a Wilcoxon test, under three different models of microsatellite evolution, the infinite allele model (IAM), the stepwise mutation model (SMM) and the two-phased model of mutation (TPM). Secondly, a graphical method was used that assumes that fewer alleles are found at lower

Table I. Relationship coefficients (R% ± SD) within and between regions in the years 1990–1999 and 2000–2006. The numbers of animals in each region are shown in parenthesis. The regions are numbered as follows: Reykjavik (01), Kjosarsysla (16), Borgarfjordur (36), Dalasysla (38), Vestur-Hunavatnssysla (55), Austur-Hunavatnssysla (56), Skagafjordur (57), Eyjafjordur (65), Sudur-Þhingeyjarsysla (66), Nordur-Þingeyjarsysla (67), Nordur-Mulasysla (75), Sudur-Mulasýsla (76), Austur-Skaftafellssysla (77) and Arnessysla (87).

		01	16	36	38	55	56	57	65	66	67	75	76	77	87
01	1990–1999 (15)	5.3±12.0													
	2000–2006 (13)	39.7±10.4													
16	1990–1999 (10)	3.0±7.3	5.1±10.3												
	2000–2006 (12)	19.1±4.7	32.7±8.2												
36	1990–1999 (91)	0	0	3.5±7.7											
	2000–2006 (147)	0.9±1.3	0.7±1.1	6.3±7.2											
38	1990–1999 (35)	0	0	0.3±1.8	2.2±6.4										
	2000–2006 (18)	0	0	0	9.7±7.9										
55	1990–1999 (9)	0	0	0	0	24.2±13.9									
	2000–2006 (0)	–	–	–	–	–									
56	1990–1999 (6)	0	0	0	0	0	13.6±18.6								
	2000–2006 (0)	–	–	–	–	–	–								
57	1990–1999 (93)	1.5±5.4	1.4±4.5	0	0	0.9±1.5	0	6.6±11.6							
	2000–2006 (31)	1.4±2.9	1.1±2.3	6.1±0.2	0	–	–	9.3±11.4							
65	1990–1999 (52)	5.0±8.9	4.8±7.0	0	0	0	0	2.5±6.0	10.4±10.8						
	2000–2006 (56)	4.7±4.7	4.0±3.8	0.2±0.6	0	–	–	0.3±0.9	2.5±5.4						
66	1990–1999 (99)	4.4±8.1	4.6±6.8	0	0	0	0	2.3±5.6	8.0±8.3	13.8±14.9					
	2000–2006 (16)	11.9±9.3	10.8±7.9	0.5±1.0	0	–	–	0.8±2.1	2.9±3.9	17.4±18.7					
67	1990–1999 (34)	8.0±13.6	7.8±10.6	0	0	0	0	4.1±9.3	12.7±11.6	11.7±11.3	21.2±17.9				
	2000–2006 (48)	13.4±13.6	10.9±10.7	0.6±1.4	0	–	–	1.1±3.6	2.8±4.5	7.6±10.6	13.8±20.0				
75	1990–1999 (20)	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			
	2000–2006 (0)	–	–	–	–	–	–	–	–	–	–	–			
76	1990–1999 (4)	0	0	0	0	0	0	0	0	0	0	0	14.7±2.2		
	2000–2006 (0)	–	–	–	–	–	–	–	–	–	–	–	–		
77	1990–1999 (11)	0	0	0	0	0	0	0	0	0	0	0	0	15.2±11.5	
	2000–2006 (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	
87	1990–1999 (120)	3.0±4.4	1.3±2.6	0.1±0.9	0	0	0	0.8±2.4	2.4±3.6	2.2±3.4	3.8±5.3	0.2±0.6	0	0	7.5±12.1
	2000–2006 (39)	5.3±2.2	4.2±1.7	3.2±5.1	20.0±1.3	–	–	0.4±0.8	1.2±1.3	3.0±2.6	3.6±4.0	–	–	–	21.5±13.0

Table II. Diversity indices calculated for fifteen microsatellite markers. Marker name, number of observations (N_{obs}), major allele frequency (MAF), number of genotypes (N_G), numbers of observed alleles (N_{OA}), observed (H_O) and expected (H_E) heterozygosity, and polymorphism information content (PIC). Markers recommended by the FAO/ISAG are marked with an asterisk.

Marker	N_{obs}	MAF	N_{OA}	N_G	H_O	H_E	PIC
CSRD247*	49	0.96	2	2	0.082	0.078	0.075
ILSTS08	51	1.00	1	1	0.000	0.000	0.000
ILSTS19	51	1.00	1	1	0.000	0.000	0.000
ILSTS087*	51	0.54	4	5	0.529	0.531	0.427
INRA023*	50	1.00	1	1	0.000	0.000	0.000
INRA172*	51	0.60	2	3	0.490	0.481	0.365
INRA063*	51	0.79	2	3	0.294	0.327	0.274
MAF065*	52	0.85	2	3	0.192	0.260	0.226
McM527*	50	0.98	2	2	0.040	0.039	0.038
OarFCB11	52	1.00	1	1	0.000	0.000	0.000
OarFCB20*	51	1.00	1	1	0.000	0.000	0.000
SRCRSP23*	51	0.60	4	5	0.471	0.510	0.415
SRCRSP5*	50	1.00	1	1	0.000	0.000	0.000
SRCRSP8*	49	0.72	2	3	0.388	0.399	0.320
INRA006	51	0.91	2	2	0.176	0.161	0.148
Mean	**50.7**	**0.86**	**1.87**	**2.27**	**0.177**	**0.186**	**0.153**

frequency classes (0.001–0.100) than in one or more intermediate frequency classes (0.101–0.900) in recently bottlenecked populations (Luikart et al., 1998). Both approaches were carried out using the computer software program BOTTLENECK (Piry et al., 1999) performing 1000 replicates (Cornuet & Luikart, 1996).

For estimation of N_e using microsatellite data the software LDNe 1.31 was applied, based on linkage disequilibrium (Waples & Do, 2008).

Mitochondrial DNA sequencing and data analysis

A 598 base pair segment, spanning positions 15,652–16,251 of the mtDNA D-loop, was amplified and sequenced using primers ChirDL-F2 (5′-CGT GTA TGC AAG TAC ATT AC-3′) and ChirDL-R1 (5′-GAT GGA CTA ATG ACT AAT CAG-3′). For PCR amplification, a 25μL PCR reaction was carried out using 12.5 μL of Taq 2 × Master Mix (New England BioLabs) which included 0.4 mM dNTPs, 50 U/ml Taq polymerase and 3.0 mM $MgCl_2$, plus 2 μL of 25 mM $MgCl_2$, 1 μL of each primer (10 pmol each), and 1 μL of genomic DNA (dilution of 10 ng/μL).

The PCR amplification was done on a Px2 Thermal Cycler (Thermo Electron Corporation) using the following setup: An initial denaturation at 96°C for 4 min, followed by 35 cycles of denaturation at 96°C for 30 sec, annealing at 53°C for 45 sec and an extension at 72°C for 1.5 min. 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) according to the manufacturer's recommendations and the DNA eluted in 40 μL of elution buffer. The purified PCR product was checked on a 1.5% agarose gel. For sequencing 0.5 μL of the appropriate primer (ChirDL-F2 or ChirDL-R1) was added to 10 μL of each sample and sequenced at MWG-Biotech (www.eurofinsdna.com). The D-loop sequences are deposited in the NCBI GenBank (www.ncbi.nlm.nih.gov/GenBank) under accession numbers JQ045384-JQ045426.

The mtDNA D-loop diversity was examined by comparing sequencing results to sequences available from NCBI GenBank. Based on D-loop sequencing a neighbour-joining tree was constructed with data from previous studies of known mitochondrial haplogroups (A, B, C, D, F and G), mtDNA D-loop sequence from wild goat *Capra ibex* and data from selected North European goat breeds.

Results

Population size

In the year 2008, the Icelandic goat population consisted of 45 herds with a total of 521 winterfed goats (Figure 1). The population size has gone from 818 animals in 1703 to 655 animals in 2009, with bottlenecks of 62 goats in 1885, 86 goats in 1893 and 99 goats in 1896. From the year 1896 a steady increase in population size is seen until the population peaks at 2983 animals in 1930. The population decreased rapidly in the following three decades to only 87 animals in the year 1962, after which time the population size has grown steadily (Figure 2).

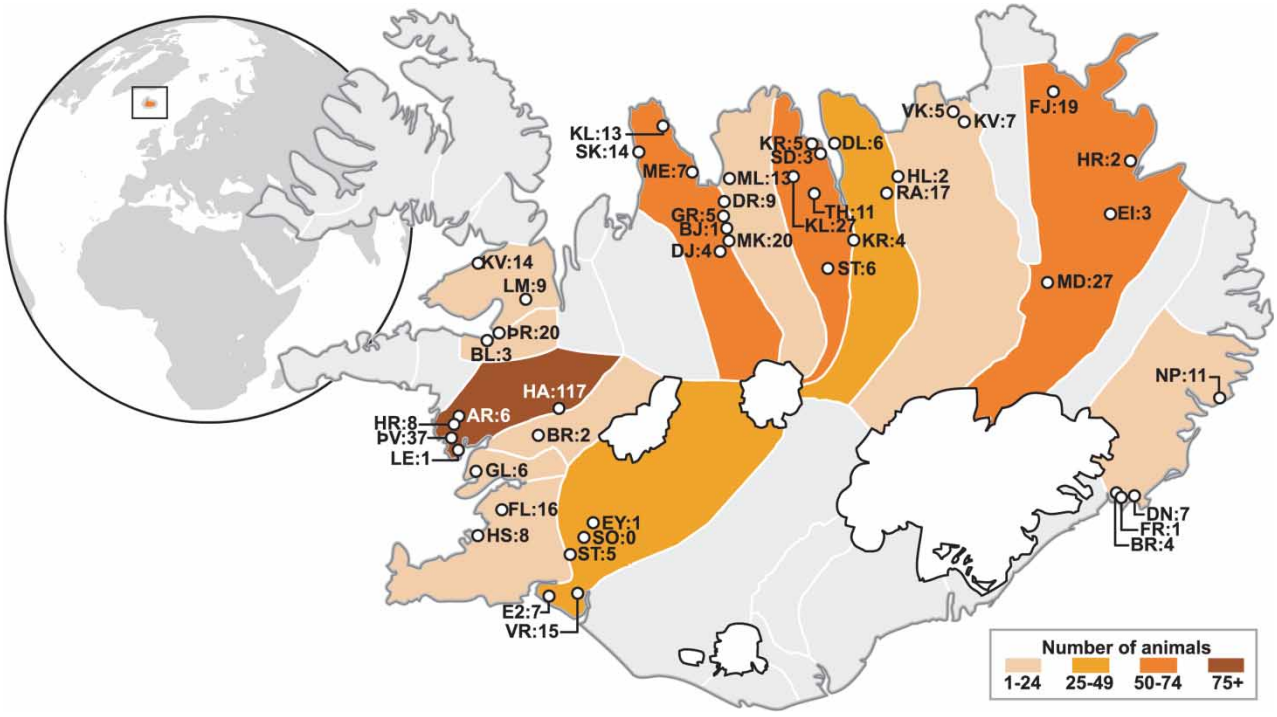

Figure 1. Geographical distribution of goats in 2008 with individual isolation zones colour coded based on total number of animals. Farm name is given as abbreviation followed by number of goats at each location. Iceland's location in a global perspective is shown in the inset.

Pedigree completeness and trend in inbreeding

The completeness of the pedigree for all animals in 2006 was 38.7% with the highest value, 47.3%, seen in the year 1997 (Figure 3). Only eight animals in the data-set had information available for all ancestors for five generations, that is a PEC5 value equal to 1. The mean inbreeding coefficient was calculated within birth years for all animals and animals with different PEC5 indices. Goats with PEC5 ≥ 0.24, ≥ 0.50, ≥ 0.70 and ≥ 0.80 were first recorded in 1974 ($n = 1$), 1978 ($n = 1$), 1981 ($n = 1$) and 1984 ($n = 2$), respectively (Figure 4a). In 2006, the number of goats recorded with PEC5 ≥ 0.24, ≥ 0.50, ≥ 0.70 and ≥ 0.80 was 38, 24, 10, and 6, respectively. The highest number for all PEC5 indices was in 1989 with $n = 63$ for PEC5 ≥ 0.24, $n = 43$ for PEC5 ≥ 0.50, $n = 32$ for PEC5 ≥ 0.70, and $n = 20$ for PEC5 ≥ 0.80 (Figure 4a and data not shown).

Average level of inbreeding for all animals in 2006 was 10.5%. For PEC5 ≥ 0.24, ≥ 0.50, ≥ 0.70, ≥ 0.80 the inbreeding was 15.9%, 19.3%, 31.5% and 50.4%, respectively. Inbreeding was first

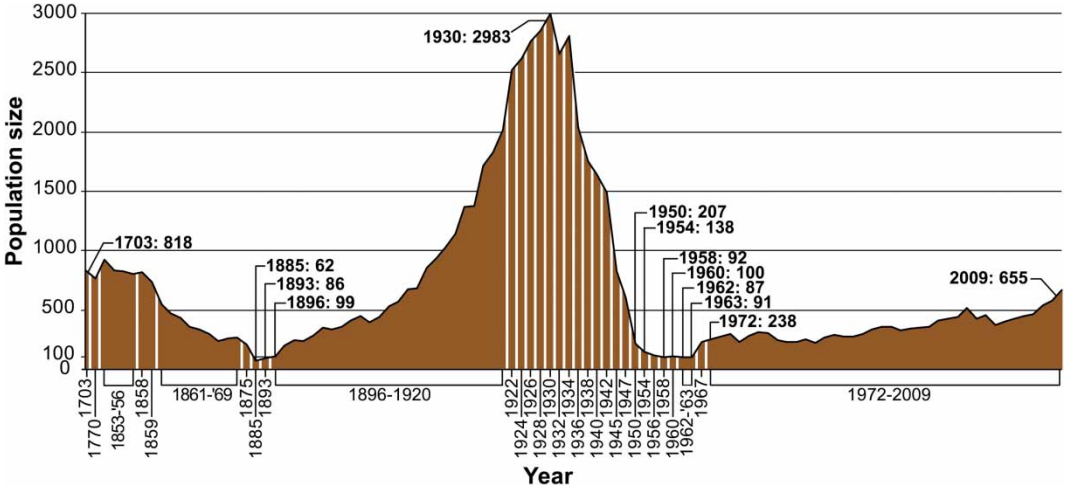

Figure 2. Development in population size in the years 1703–2008 with years of special interest labeled specially followed by the exact number of goats. The available data is at times non-continuous, indicated by bars.

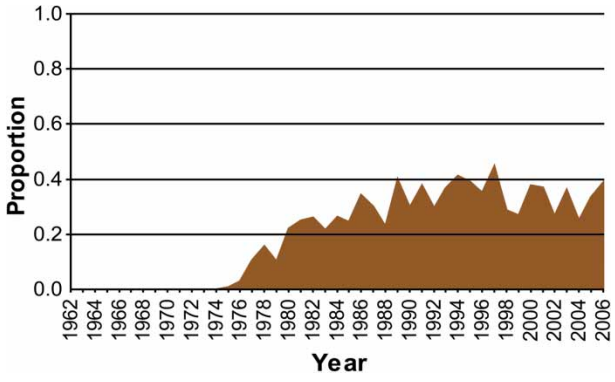

Figure 3. Available pedigree data for five generations for the population in the years 1962–2006.

detected in 1974 for all animals (0.5%) and animals with PEC5 $\geq$0.24 (25%), in 1978 for animals with PEC5 $\geq$0.50 (18.8%), in 1981 for animals with PEC5 $\geq$0.70 (21.5%) and in 1984 for PEC5 $\geq$0.80 (45.8%). Highest inbreeding was calculated in 1985 with PEC5 $\geq$0.80 (64.4%) (Figure 4b).

Inbreeding is first detected for animals born in 1974 with 2% of animals inbred where as in 2006 that proportion had increased to 62.5% or 35 out of 56 animals born in that year. The highest proportion of inbred individuals, 70.9%, was seen in the birth cohort of 1980 (39 inbred out of 55 individuals). The highest individual inbreeding observed was 71.1%, with the ten most highly inbred goats in the period 1962–2006 ranging from 56.1–71.1% inbreeding. Two does born in 1986 and 1987 were 71.1% inbred and had a PEC5 index of 0.93 and all of the ten most inbred goats had PEC5 $\geq$0.87 (data not shown).

Individual genetic contributions to the population were calculated for two birth years, 2002 and 2006. Individual 1994287120 contributed the most in 2002 (9.5%) and individual 2004136001 contributed the most in 2006 (16.5%). Four individuals are on the top 10 lists for both years (data not shown).

Effective population size and coefficient of relationship

Generation length for father–son, father–daughter, mother–son, and mother–daughter was found to be 2.9 years, 3.5 years, 3.1 years and 4.5 years,

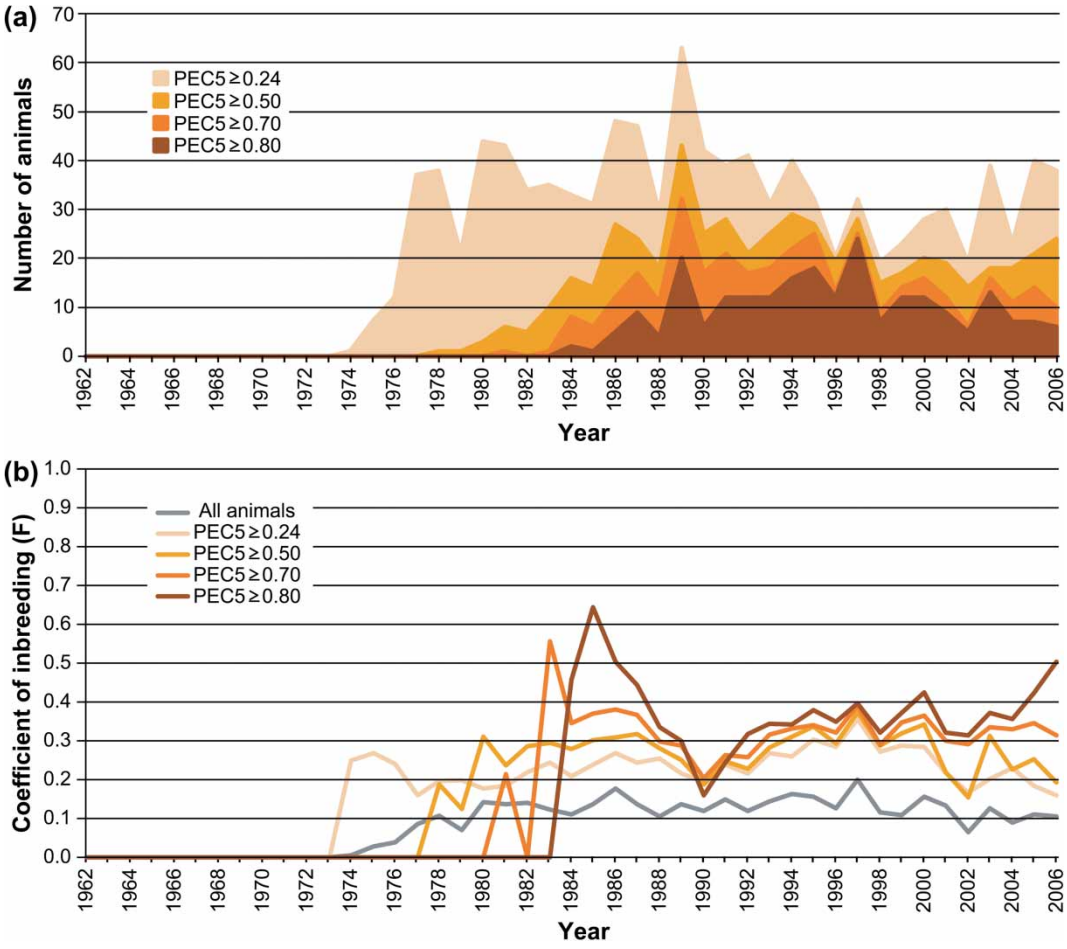

Figure 4. Pedigree completeness and number of animals with (a) number of individuals available with different levels of pedigree completeness (PEC) and (b) the development in inbreeding (F) by birth year 1962–2006.

respectively. Average generation length (L) for all animals born 1962–2006 was 3.5 years. The rate of increase in inbreeding per year was found to be $\Delta F_y = 3\%$ ($P < 0.001$) and per generation $\Delta F = 9.9\%$ ($P < 0.001$). Based on these the effective population size (N_e) was estimated 5.1 animals.

To better understand the population structure the coefficient of relationship (R) was calculated from pedigree data within and between regions (Table I) for the time periods 1990–1999 and 2000–2006. R within regions increased in almost all cases between the two periods, except for the Eyjafjordur (area 65) and Nordur-Thingeyjarsysla (area 67) where R decreased from 10.4% to 2.5% and from 21.2% to 13.8%, respectively. The highest increase in the coefficient of relationship within regions was in Reykjavik (area 01) and Kjosarsysla (area 16) where R increased from 5.3% to 39.7% and from 5.1% to 32.7%, respectively. The coefficient of relationship was higher within regions than between areas, except for regions 01 and 67 (1990–1999) and regions 38 and 87 (2000–2006).

Phenotypic diversity

Considerable diversity is found in coat colour and patterning within the Icelandic goat population, including solid white/tan, solid gray, four types of piebald, as well as the badger-face coat colour phenotype. Also, a small part of the population is polled (Figure 5a–f). The three most common coat colour patterns are black piebald (Figure 5b and e), grey piebald (Figure 5b) and white or tan (Figure 5a). The increasing size of the population has led to an increase of all coat colors and patterns, except for the solid gray phenotype (Figure 5g). The number of polled individuals has also increased although they are only found at a single farm (HA in Figure 1). The badger-face colour pattern with tan most often accompanies the polled phenotype.

Microsatellite and mtDNA diversity

For microsatellite analysis a total of 52 samples were analysed, or approximately 8% of the population,

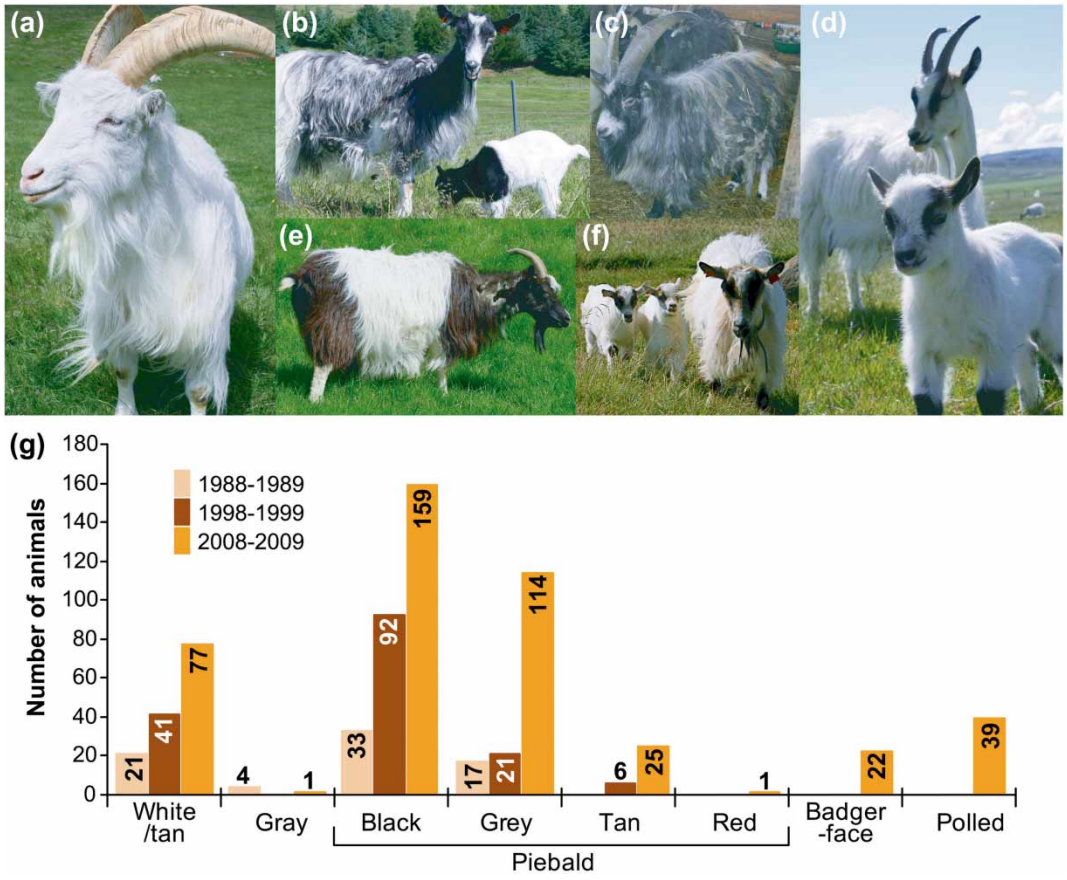

Figure 5. Examples of coat colour variation found within the Icelandic goat population with (a) white/tan, (b) gray piebald polled doe with black piebald kid, (c) gray piebald, (d) white, badger-faced doe with badger-faced kid, (e) black piebald, (f) polled tan piebald badger-faced doe with kids, and (g) a summary of coat colour variation and number of polled animals within the goat population at three time periods, 1988––1989, 1998–1999, and 2008–2009.

from six farms or around 13% of Icelandic goat farms, revealing 27 alleles across 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 (Table II). Additionally, two of the dimorphic loci (CSRD247 and McM527) had a frequency of the more common allele exceeding 0.950. Several more alleles occurred at low frequencies (Table II). The mean observed heterozygosity (H_O) was 0.177 or 0.364 when excluding monomorphic markers, and mean expected heterozygosity (H_E) was 0.186, or 0.307 when excluding monomorphic markers. Two loci (ILSTS087 and SRCRSP23) showed significant deviations from HWE (exact test $P<0.001$) and when examined over all loci the results showed significant deviation from HWE (exact test $P<0.001$). Based on microsatellite markers the inbreeding coefficient (F_{IS}) over all loci was estimated as 2.6% and N_e in the range of 4.1–8.8 individuals (lower and upper 95% confidence limits 2.2 and 21.6, respectively). Analysis of heterozygosity excess/deficiency and the mode-shift test showed no evidence of recent bottlenecks (data not shown).

Sequencing of the mitochondrial DNA D-loop identified 12 variable sites giving rise to three D-loop haplotypes. Two of the haplotypes [represented by samples ChIce_030 (GenBank accession number JQ045407) and ChIce_050 (GenBank accession number JQ045413)] varied only in one polymorphic site at nucleotide position 15,871. A neighbour-joining tree based on D-loop alignments of Icelandic sequences, sequences of defined mitochondrial haplogroups (A, B, C, D, F and G), sequences from selected North European goat breeds and a D-loop sequence from *Capra ibex* gives a tree where all Icelandic sequences and most of the other North-European goats belong to haplogroup A (data not shown).

Discussion

Studies have shown that when a large proportion of pedigree information is missing the resulting calculations may underestimate the level of inbreeding and overestimate the effective population size (Boichard et al., 1997; Lutaaya et al., 1999). The amount of pedigree data available for the Icelandic goat population is low for all years with considerable fluctuations. For example, PEC5 was only 38.7% in the year 2006 (Figure 3) and only eight animals in the whole data-set (a total of 2240 individuals) had a complete pedigree for five generations. This means that the levels of inbreeding calculated here are most likely underestimates, although it is difficult to say to what extent. This is supported by the fact that increased inbreeding is associated with more complete pedigree data (Figure 4) and that the animals with the highest inbreeding coefficients all had relatively high levels of pedigree completeness (PEC5 ≥ 0.87) suggesting that more complete pedigree data would reveal even higher levels of overall inbreeding than seen here. Should this hold true then the estimated effective population size of $N_e = 5.1$ based on pedigree data is also most likely an over-estimation.

The proportion of inbred animals in the population is estimated at 62.5% in the year 2006 and the increase in inbreeding per generation, ΔF equal to 9.9% ($P < 0.001$), is tenfold that recommended by FAO. In 2006, the inbreeding coefficient calculated for animals with PEC5 ≥ 0.70 was 30.5%, compared to 26% in the period 1977–1992 (Adalsteinsson et al., 1994). Previous results for the Icelandic goat breed have shown that a 10% increase in F resulted in 2.8% decrease in fertility, 0.8% decrease in total number of kids born and 2.6% decrease in the number of kids born alive, underlining the necessity of reducing the rate of inbreeding in the Icelandic goat population (Adalsteinsson et al., 1994).

Threatened populations are vulnerable to the effects of both genetic drift and inbreeding, particularly when gene flow is low and the effective population size is small. Estimates of N_e provide important information on the status of endangered populations and serve as indicators of genetic diversity. Breeds with an inbreeding rate per generation higher than 1%, equivalent to a N_e lower than 50 individuals, are considered to be in a critical state. It has been suggested that N_e should be at least 50 to prevent inbreeding depression from becoming a serious problem and that N_e should be in the range of 500–5000 to retain genetic diversity and thereby the long-term evolutionary potential of the population (Franklin & Frankham, 1998). The N_e estimates for the Icelandic goat population of 5.1 animals based on pedigree data and 4.1–8.8 individuals based on microsatellite markers are far below recommendations, underlining the breed's long-term problems with population size. These values are among the lowest N_e values reported in the literature for domestic breeds. The lowest N_e value found based on pedigree data was $N_e = 14$ for the Japanese Black cattle, a population of 0.53 million reproductive cows (Nomura et al., 2001), $N_e = 23.3$ for the Alentejana cattle with a population of 12000 breeding cows, and $N_e = 25.1$ for the Malhado de Alcobaca pigs a population of 160 breeding sows (Gama et al., 2008). For N_e based on molecular markers the observed value is similar to that found for Chillingham cattle with N_e around 8, an extensively studied

genetically uniform breed which has remained viable despite at least 300 years of total inbreeding (Visscher et al., 2001).

The high genetic contribution of few ancestors leads to increased inbreeding and is detrimental to the long-term viability of a population (Woolliams & Thompson, 1994). The high contribution of buck 2004136001 in 2006 is the result of his extensive use in the years 2005 and 2006, when he fathered 17% and 19.2% of the kids born, respectively. The buck 2004136001 comes from the biggest herd, a herd that counts over one hundred does. This underlines two of the problems facing those involved in goat breeding in Iceland; that is lack of breeding advice and the lack of options when selecting bucks for the next generation. It has been pointed out that the sire breeding part of a population largely governs the rate of inbreeding (Goddard & Smith, 1990; Rochambeau et al., 2000) and simulation studies have shown that breeding schemes that use more sires result in lower rate of inbreeding (Korpiaho et al., 2002).

Genetic diversity measures based on microsatellite markers confirmed the poor status of the Icelandic goat breed. The mean number of alleles equal to 1.8, with numbers varying from one to four alleles per locus, is similar to results seen for the Japanese Mishima Island cattle population where the mean number of alleles (MNA) for 21 loci was 1.85 (Nagamine et al., 2008). Genetic diversity (GD or H_E) in the Icelandic goat population is low with H_E equal to 0.307 for nine polymorphic loci, and 0.185 including fixed loci. This level of genetic diversity is among the lowest reported from analyses of microsatellites in mammals, much lower than for example results for the Grisons Striped goat breed ($H_E = 0.670$ for 30 loci and MNA = 6.5) and the Girgentana ($H_E = 0.656$ for 30 loci and MNA = 5.7) both of which are listed by FAO with endangered risk status (Canon et al., 2006) and similar to results for the Chillingham cattle (Visscher et al., 2001) and the Japanese Mishima Island cattle (Nagamine et al., 2008). These very low values raise the obvious question whether the markers used are applicable for the analysis of genetic diversity in goat populations. The markers used here have all been used previously in goat population studies and have been shown to be well suited for genetic diversity analysis (e.g. Canon et al., 2006), suggesting that the results observed here truly reflect the poor status of genetic diversity within the Icelandic goat breed.

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, one in 1885 and another in 1962, when the population was reduced to 62 and 87 animals, respectively. Methods based on heterozygosity excess do not reveal evidence of recent bottlenecks despite the breed's population history. This might be explained by substructures within the population due to the fragmentation (Cornuet & Luikart, 1996). Studies of *Capra ibex* with known bottlenecks gave similar results, but when the population was separated into two geographic sub-populations the results showed a significant bottleneck signature (Maudet et al., 2002). This was not possible here due to the low number of individuals from different areas.

The inbreeding coefficient for the Icelandic goat population (mean $F_{IS} = 2.6\%$) estimated from the microsatellite data was much lower than that 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 those that 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 et al., 2004). Furthermore, both theoretical and empirical data 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).

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 at one polymorphic site. The genetic diversity of goat mtDNA has been studied on a large scale and has shown high levels of diversity with over 1500 haplotypes reported falling into six haplogroups (Luikart et al., 2001; Naderi et al., 2007), supporting the idea that the low observed diversity within the Icelandic goat population reflects the status of the breed rather than limitations of the pedigree data or the microsatellite markers used.

In order to better understand the structure of the population, relationship (R) within and between regions was estimated based on pedigree information. Fragmentation of the population was suspected as the country is divided into isolation zones, put in place to control the spread of diseases, which limits movement of goats across regions. The results reflect this fragmentation as the relationship within regions is higher than between regions. Although the levels of inbreeding reported here are most likely underestimates due to low data quality, the underestimation should apply equally to the relationships within and between regions, making the differences in R the

point of interest rather than the absolute values. Based on these results we conclude that reducing inbreeding within zones by breaking the isolation of herds through for example artificial insemination could be an important step that would also add extra protection to the breed as semen could be stored for future use. Analysis of population structure using molecular markers was unsuccessful due to low levels of genetic diversity and low numbers of samples from different regions (data not shown). Although analysis of population structure could be reattempted using more markers, possibly relying on methods such as genome-wide high-density SNP arrays or high-throughput whole genome sequencing, possibly revealing signs of population structure as well as giving information on levels of homozygosities and relations to other breeds, it is worth mentioning that no structure has been detected in other Icelandic livestock breeds such as the Icelandic cattle (Asbjarnardottir et al., 2010) and the Icelandic horse (unpublished results).

Despite low estimates of genetic diversity the Icelandic goat breed harbors considerable variation in coat colour, including solid white or tan, solid gray, as well as four types of piebald. The solid brown colour, once found in the population, is believed to have been lost at least two decades ago. With at least seven coat colour patterns identified it is not obvious how to explain the discrepancy between phenotypic and genotypic variation. One possible explanation is that although the Icelandic goat population has gone through bottlenecks in its history it has been bred in many small herds, each with own characteristics and without a breed description, but such descriptions could have limiting effects on phenotypic variation.

Another interesting discrepancy between the results presented here and the current state of the breed is the fact that despite the low levels of genetic diversity the negative effects associated with inbreeding, such as low fertility, stillbirths, and high frequency of congenital defects, are seen only in a single heavily inbred herd (unpublished results). Although the importance of purging in protecting small endangered populations from extinction is debated (Frankham et al., 2001) the results presented here raise the question whether purging of deleterious alleles, probably occurring in many small inbred herds, may have left the Icelandic goat breed protected to some extent from the deleterious effects of inbreeding.

In the light of ever decreasing global genetic diversity of domestic animals it is of great importance to protect unique breeds such as the Icelandic goat from further genetic erosion and secure a sustainable future for this population that is believed to have existed in isolation for over 1100 years.

In view of the results presented here, showing high levels of inbreeding, population fragmentation, and low levels of genetic diversity as seen with molecular markers, an important step in protecting the breed would be to improve the pedigree records so to be able to better monitor the rate of inbreeding and direct the breeding efforts in the right direction. Another possibility would be to use more markers, preferably high-density SNP arrays or whole genome sequencing, to resolve the genetic relationship between individuals, both within and between regions. Also, mating programs aimed at selecting the best suited parents to the next generation to minimize the level of inbreeding should be applied. This requires increased effort from the breeders to record pedigree data with more precision in the future as well as dedication from those institutions that can advise on breeding strategies as a way of minimizing the effects of inbreeding. The breeding population should include all animals and the isolation of subgroups needs to be broken. This could possibly be done through an increased emphasis on semen collection and the use artificial insemination as a way to better steer the breeding effort. This would additionally open up the possibility of semen storage as a backup for genetic material for future generations which would give breeders more choices in their breeding work.

Further studies to evaluate the genetic diversity of the goat breed are necessary, preferably relying on high-density markers, but better pedigree records are also needed for an ongoing revitalization of the Icelandic goat breed. Such work should be done in the context of a long-term conservation plan based on a detailed population viability analysis. As a follow up to the work presented here the first steps have been taken towards semen collection, long-term semen storage and artificial insemination. Despite the welcome increase in population size the Icelandic goat breed has experienced in the last 10 years and the positive signs of a possible commercial utilization, it is important to increase the population size even further, as a larger population is needed for product utilization and to sustain increased emphasis on product development.

The low levels of genetic diversity observed here with the lack of clear signs of associated inbreeding depression within the Icelandic goat population might suggest that purging of deleterious alleles in many small sub-populations, rather than in a single breeding unit, might offer an efficient way of purging and at the same time minimizing the possibility of extinction.

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References

Adalsteinsson, S. (1981). Origin and conservation of farm animal populations in Iceland. *Journal of Animal Breeding and Genetics*, 98, 258–264.

Adalsteinsson, S., Dyrmundsson, O. R., Bjarnadottir, S. & Eythorsdottir, E. (1994). Skyldleikarækt í íslenskum geitum (in Icelandic). *Icelandic Agricultural Sciences*, 8, 99–105.

Asbjarnardottir, M. G., Kristjansson, T., Jonsson, M. B. & Hallsson, J. H. (2010). Analysis of genetic diversity and population structure within the Icelandic cattle breed using molecular markers. *Acta Agriculturae Scandinavica Section A-Animal Science*, 60(4), 203–210.

Balloux, F., Amos, W. & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, 13, 3021–3031.

Boichard, D. (2002). Pedig: A Fortran package for pedigree analysis suited for large populations. *Proceedings of 7th World Congress on Genetics Applied to Livestock Production*, August 19, Montpellier, France, Institut National de la Recherche Agronomique (INRA).

Boichard, D., Maignel, L. & Verrier, E. (1997). The value of using probabilities of gene origin to measure genetic variability in a population. *Genetics Selection Evolution*, 29(1), 5–23.

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32(3), 314–331.

Canon, J., Garcia, D., Garcia-Atance, M. A., Obexer-Ruff, G., Lenstra, J. A., Ajmone-Marsan, P., Dunner, S. & Econogene Consortium. (2006). Geographical partitioning of goat diversity in Europe and the Middle East. *Animal Genetics*, 37(4), 327–334.

Cornuet, J. M. & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, 144(4), 2001–2014.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics* (4th edn). Harlow: Essex: Longmans Green.

Fernandez, J., Villanueva, B., Pong-Wong, R. & Toro, M. A. (2005). Efficiency of the use of pedigree and molecular marker information in conservation programs. *Genetics*, 170(3), 1313–1321.

Frankham, R. (2005). Stress and adaptation in conservation genetics. *Journal of Evolutionary Biology*, 18(4), 750–755.

Frankham, R., Gilligan, D. M., Morris, D. & Briscoe, D. A. (2001). Inbreeding and extinction: Effects of purging. *Conservation Genetics*, 2(1995), 279–285.

Franklin, I. R. & Frankham, R. (1998). How large must populations be to retain evolutionary potential? *Animal Conservation*, 1(1), 69–70.

Gama, L. T., Carolino, N. & Vicente, A. (2008). Monitoring of livestock breeds at risk in Portugal. Accessed 30 October 201, available at: www.save-foundation.net/ELBARN/pdf/Gama_Livestock%20risks%20Portug.pdf

Goddard, M. G. & Smith, C. (1990). Optimum number of bull sires in dairy cattle breeding. *Journal of Dairy Science*, 73(4), 1113–1122.

Hoffmann, I., Marsan, P. A., Barker, J. S. F., Cothran, E. G., Hanotte, O., Lenstra, J. A., Milan, D., Weigend, S. & Simianer, H. (2004). *New MoDAD Marker Sets to be Used in Diversity Studies for the Major Farm Animal Species: Recommendations Of A Joint ISAG/FAO Working Group.* Accessed 12 September 2009. Available at: http://lprdad.fao.org/cgi-bin/getblob.cgi?sid=574c5790c205740a10a9b39dc599fead,50005882

Korpiaho, P., Stranden, I. & Mäntysaari, E. A. (2002). Mating strategies in a small nucleus scheme. *Proceedings of 7th World Congress Genetics Applied to Livestock Production*, 19 August, Montpellier, France, Institut National de la Recherche Agronomique (INRA).

Lacy, R. C. (1995). Clarification of genetic terms and their use in the management of captive populations. *Zoo Biology*, 14(6), 565–577.

Liu, K. & Muse, S. V. (2005). PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics Applications Note*, 21(9), 2128–2129.

Luikart, G., Allendorf, F. W., Cornuet, J. M. & Sherwin, W. B. (1998). Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, 89(3), 238–247.

Luikart, G., Gielly, L., Excoffier, L., Vigne, J. D., Bouvet, J. & Taberlet, P. (2001). Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proceedings of the National Academy of Sciences of the United States of America*, 98(10), 5927–5932.

Lutaaya, B. E., Misztal, I., Bertrand, J. K. & Mabry, J. W. (1999). Inbreeding in populations with incomplete pedigrees. *Journal of Animal Breeding and Genetics*, 116(6), 475–480.

MacCluer, J. W., Boyce, A. J., Dyke, B., Weitkamp, L. R., Pfenning, D. W. & Parsons, C. J. (1983). Inbreeding and pedigree structure in Standardbred horses. *Journal of Heredity*, 74(6), 394–399.

Maudet, C., Miller, C., Bassano, B., Breitenmoser-Wursten, C., Gauthier, D., Obexer-Ruff, G., Michallet, J., Taberlet, P. & Luikart, G. (2002). Microsatellite DNA and recent statistical methods in wildlife conservation management: Applications in Alpine ibex [Capra ibex (ibex)]. *Molecular Ecology*, 11(3), 421–436.

Meuwissen, T. & Luo, Z. (1992). Computing inbreeding coefficients in large populations. *Genetics Selection Evolution*, 24, 305.

Naderi, S., Rezaei, H. R., Taberlet, P., Zundel, S., Rafat, S. A., Naghash, H. R., El-Barody, M. A. A., Ertugrul, O., Pompanon, F. & Econogene Consortium. (2007). Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. *Plos One*, 2(10), e1012.

Nagamine, Y., Nirasawa, K., Takahashi, H., Sasaki, O., Ishii, K., Minezawa, M., Oda, S., Visscher, P. M. & Furukawa, T. (2008). Estimation of the time of divergence between Japanese Mishima Island cattle and other cattle populations using microsatellite DNA markers. *Journal of Heredity*, 99(2), 202–207.

Nomura, T., Honda, T. & Mukai, F. (2001). Inbreeding and effective population size of Japanese Black cattle. *Journal of Animal Science*, 79(2), 366–370.

Peakall, R. & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295.

Piry, S., Luikart, G. & Cornuet, J. M. (1999). Bottleneck: A computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity*, 90, 502–503.

Rochambeau, H., de, Fournet-Hanocq, F. & Khang, J. V. T. (2000). Measuring and managing genetic variability in small populations. *Annales de Zootechnie*, 49(2), 17.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106.

Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E. & McEwan, J. C. (2004). Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: Theoretical expectations and empirical data. *Heredity*, 93, 255–265.

Sørensen, M. K., Sørensen, A. C., Baumung, R., Borchersen, S. & Berg, P. (2008). Optimal genetic contribution selection in Danish Holstein depends on pedigree quality. *Livestock Science*, 118(3), 212–222.

Taberlet, P., Valentini, A., Rezaei, H. R., Naderi, S., Pompanon, F., Negrini, R. & Ajmone-Marsan, P. (2008). Are cattle, sheep and goats endangered species? *Molecular Ecology*, 17(1), 275–284.

Visscher, P. M., Smith, D., Hall, S. J. G. & Williams, J. L. (2001). A viable herd of genetically uniform cattle. *Nature*, 409(6818), 303.

Waples, R. S. & Do, C. (2008). LDNE: A program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, 8(4), 753–756.

Woolliams, J. A. & Thompson, R. (1994). A theory of genetic contributions. *Proceedings of 5th World Congress Genetics Applied to Livestock Production*, August 7–12, Guelph, Ontario, Canada.