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Analys av inavel hos svenska gotlandsruss baserad på härstamningsinformation och molekylärgenetiska markörer

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Table of contents

Abstract	4
Sammanfattning	4
Introduction	5
Literature study	5
Background	5
Breed description.....	5
Breed history	5
Breeding Gotland ponies.....	7
Breeding statistics	7
Conservation strategies	8
Population genetics	8
Inbreeding.....	8
Methods to estimate inbreeding	10
Genealogical methods	10
Molecular markers.....	11
Similar studies	12
Pedigree analysis in other horse breeds.....	12
Molecular analysis in other horse breeds	13
This study	15
Material and methods	15
Pedigree data	15
Pedigree analysis	15
Molecular data.....	17
Results	18
Population facts	18
Inbreeding and pedigree completeness	19
Generation interval	21
Effective population size	21
F _{IS} and heterozygosity based on molecular marker information.....	21
Hardy-Weinberg equilibrium	23
Probability of exclusion (PE) and polymorphic information content (PIC)	23
Discussion	24
Data	24
Population facts	24
Effective population sizes	25
Genealogical results	25
Molecular results	27
Correlation between genealogical and molecular information	29
Conclusions	30
Acknowledgments	30
References	31

Abstract

In this study inbreeding in the Swedish indigenous breed Gotland pony has been investigated using traditional genealogical methods and microsatellite markers. Since the breed is classified as endangered-maintained, it is valuable to know the inbreeding status and population structure. Pedigree data was very complete with PEC-values for some horses above 0.8 already during the first decades of the 1900's. The average inbreeding coefficient was 0.11 for horses born 1996 to 2005. The average increase in inbreeding was 0.75 % per generation since 1985. The average generation interval was 10.4 years. The inbreeding effective population size was 67 individuals. The effective population size based on the variance in progeny group was 235 ponies. On average, each year 10 new stallions and 81 new mares were used in breeding every year. The majority of stallions used in breeding had between 1 and 20 registered offspring.

The DNA profiles from 344 horses were analysed. In total 16 markers were considered. F_{IS} , a measure of inbreeding related to the subpopulation, was 0.014 when considering all 344 animals as a single population. The expected level of heterozygosity was 0.643 while the observed was 0.635. One marker, AHT5, was found not to be in Hardy-Weinberg equilibrium, indicating some kind of selection.

Sammanfattning

I detta arbete har inaveln inom den svenska ponnyrasen Gotlandsrusset undersökts både genom analys av härstamningsdata och via molekylärgenetiska metoder. Rasen är klassad som "hotad-bevarad" och därför är rasens inavelstatus viktig att veta. Härstamningsdata var förhållandevis komplett då PEC-värden över 0,8 förekom för en del hästar redan under de första årtiondena under 1900-talet. Medelinavelskoefficienten var 0,11 för hästar födda mellan 1996-2005. Den genomsnittliga ökningen av inavelsgraden var 0,75 % per generation för hästar födda efter 1985. Det genomsnittliga generationsintervallet var 10,4 år. Den inavelseffektiva populationsstorleken är 67 djur. Den effektiva populationsstorleken baserad på variationen i avkommegruppstorlek gav 235 djur. I genomsnitt sattes det in 10 nya hingstar och 81 ston i avel per år. Majoriteten av hingstar i avel har mellan 1 och 20 registrerade avkommor.

Vid den molekylärgenetiska analysen har DNA-information från 344 russ använts och 16 mikrosatelliter har analyserats. F_{IS} , ett mått på inavel relaterat till en subpopulation, är 0,014 för alla 344 djur. Den förväntade heterozygotigraden var 0,643, medan den observerade heterozygotigraden är 0,635. För alla 344 djur var en markör, AHT5, inte i Hardy-Weinberg jämvikt, vilket kan tyda på någon slags selektion.

Introduction

The Gotland pony is one of few indigenous horse breeds to Sweden and named after the island in the Baltic Sea from which it originates. The breed declined rapidly in number when grazing areas were transformed into farming land in the 19th century. Measures were then taken to preserve the breed from extinction. Due to the low number of founding animals, inbreeding soon became a problem and the decision was made to bring in some Welsh pony stallions to the breed. The first studbook was published in 1943, and in 1967 the breed organisation was founded. Today, about 130 stallions and approximately 600 mares are used in breeding. The number of animals born and registered each year is approximately 350 and has increased considerably since the 1960's, in part due to the fact that the ponies have found their niche as children's riding and trotting companion.

The Swedish Board of Agriculture has declared the breed to be "endangered-maintained" according to the FAO scale. This means that the breed is considered worth preserving for future generations. There are currently plans for collecting semen from pony stallions to be saved in a gene bank administered by the Board of Agriculture.

The purpose of this study was to investigate the level of heterozygosity and rate of inbreeding in the Gotland pony breed, using both traditional pedigree analysis and the modern method with microsatellite markers. Other measures such as effective population sizes were also of interest to estimate. Comparison of results from the pedigree and the microsatellite method will also be discussed.

Literature study

Background

Breed description

The Gotland pony should be a harmonious, well proportioned pony with a good breed type (Svenska Rassevelföreningen, 2009). The head should be proportional with an alert look. The back should be strong with enough space for the saddle. Legs and hoofs should be correct, and movements energetic, elastic and cover a lot of ground. Ideal height at withers is between 123 and 126 cm. Variation from 115 up to 130 cm is however accepted. All colours are allowed apart from greys, duns, tobiano, and homozygous creams.

Since the breed is of small stature it is mainly a children's pony. It is mainly used in show jumping, dressage and trotting by children and adolescents.

Breed history

Early history

The Gotland pony is an indigenous pony breed from the island of Gotland. There have been ponies on the island for thousands of years (Elmlund, 1993). The oldest reference of horses on the island is from the early 13th century (Hallander, 1989).

During the 19th century the number of ponies on the island decreased steadily when grazing areas were turned into agriculture land. The ponies had throughout times been grazing freely but now they were gathered together and sold because pasture land was scarce. Some years several hundreds of ponies were sold from the island. A group of people then started to worry that the breed would go extinct and took measure to save it.

In 1886, Mr. Wöhler bought the stallion Khediven 1, who had unknown pedigree, and used him to cover his mares. Khediven was the stallion who brought the appaloosa colour into the breed. Another widely used stallion in the beginning of the 20th century was Olle 2, a black stallion with a Gotland pony mother. Other non purebred stallions were also used to cover Gotland pony mares, but the two previously mentioned are the only ones that still can be found in pedigrees.

At the beginning of the 20th century it was estimated that 150 ponies existed on the island, primarily in the Lojsta forest (Hallander, 1989). Two studs were formed but business did not flourish and both had to stop their activities (Elmlund, 1993). When the last stud closed, the animals still owned by the stud was granted to the Swedish rural economy and agricultural society (Hushållningsällskapet) on Gotland, provided that they continued keeping a herd of Gotland ponies.

Breeding on the mainland of Sweden started in the 1920's when zoological gardens in Stockholm and Gothenburg bought some breeding animals (Elmlund, 1993). When ponies outside the island became eligible for breed evaluation (premiering) in 1961 the number of ponies steadily increased (Hallander, 1989).

Lojsta

The ponies owned by the Swedish rural economy and agricultural society was after some discussion let out in the forest of Lojsta. The land grazed by the ponies was divided into three parts, and the herd was moved around depending on season of year. There are still today a herd of ponies on Lojsta. The herd consists of about 50 mares and youngsters and each year a stallion is let in (Svenska Russavelsföreningen, 2009). Only mares born in the forest are allowed to be in the herd (Hallander, 1989).

Since the stallions used on Lojsta in the early years were used excessively, inbreeding soon became a problem. For example, in the 1940's only nine stallions were licensed for breeding (Svenska Russavelsföreningen, 2009). Two Welsh stallions were bought in the 1950's and 1960's to get unrelated blood, even though this measure was debated by breeders (Elmlund, 1989). The Welsh blood is today present in many of the ponies, however the percentage in every individual is likely to be low (Svenska Russavelsföreningen, 2009).

Cerebellar ataxia

One disease popped up due to the heavy inbreeding: cerebellar ataxia. The founder was likely the stallion Olle 2 (Elmlund, 1993). This is a recessive genetic disease causing the cerebellum to be underdeveloped. The symptoms are difficulties keeping the balance and control of the movements. Stallion carriers are continuously removed from breeding (Hallander, 1989). Since the disease is recessive and carriers undetected, some affected animals can still be born.

The Studbook and breed association

A nation-spanning breed association was formed in 1967 (Svenska Russavelsförening, 2009). Since 1975 the breed association has been a part of the Swedish pony breed society (SPAF), which in turn is subordinate to the Swedish horse breeding society (SH). The first studbook was published in 1943 by the Swedish rural economy and agricultural society on Gotland (Svenska Russavelsföreningen, 2009). The studbook was closed in 1971 for animals that do not have a purebred pedigree (Hallander, 1989).

Breeding Gotland ponies

Stallions

To get a license to use a stallion for breeding, he has to be approved by a committee appointed by the board of the Swedish horse breeding society (Svenska Hästavelsförbundet). A stallion can be approved at the earliest at 2.5 years of age (Svenska Hästavelsförbundet, Hingstreglemente, 2009). The horse has to be registered in the main section of the studbook and pass a veterinary inspection. The committee evaluates the horse on 5 judging areas: breed type; head-neck-body; legs; walk and trot. In each area a score between 1 and 10 is given, the maximum total score is 50 points. To be approved a stallion has to reach 40 points with no score below 7. Exceptions can be made but if stallions are to be approved on 38-39 points, they need to have special merits, e.g. competition results, rare pedigree or rare colour.

A stallion that has 15 offspring that are three years or older and that have been judged in some way is to be progeny tested. The stallion may then be awarded C, B, A, or ELIT, depending on how good offspring he has produced. (Svenska Hästavelsförbundet 2009).

Mares

Mares do not need to be approved by the Swedish horse breeding society to be used in breeding. However many mare owners show their mares at voluntary inspections arranged all over the country in the summer. The mares are judged on breed type, head-neck-body, legs, walk and trot. The maximum total score is 50; hence every part can be given a score of 1 to 10 (Svenska Hästavelsförbundet - Storeglemente, 2009).

Three-year-old mares reaching 40 points, with no score lower than 7 are awarded a breeding diploma. Mares without offspring or with less than 5 living offspring are evaluated on their own performances, and are thereafter awarded with G, GI or GII. Mares with 5 or more foals born alive will be judged on their progeny performance and may be awarded A, ELIT or Super-ELIT. (Svenska Hästavelsförbundet 2009).

Breeding statistics

In 2006, 107 stallions were available for breeding and they covered 564 mares (Svenska Hästavelsförbundet - Blå boken 2007, 2008). The following year 368 registered foals were born. This gives a foaling percentage of 65%. For comparison, the foaling percent for the New Forest was 69%, for the Connemara 59% and for the Welsh Pony 69%. The number of stallions available for breeding and number of covered mares in other years are shown in Table A. The average number of covered mares per stallion in 2007 was 5.7.

Table A. Number of stallions and covered mares (Blå boken 1996, Blå Boken 2007)

	1960	1970	1980	1990	1995	2000	2001	2002	2003	2004	2005	2006	2007	2008
Nr of stallions					121	125	124	120	112	117	101	107	109	
Covered mares	184	1232	795	795	629	597	565	557	563	600	560	564	623	632

In 2007, a total of 157 stallions were shown for the committee to be approved or rejected as breeding stallions or for an evaluation of their offspring (Svenska Hästavelsförbundet - Blå boken 2007, 2008). The voluntary inspection during the summer of 2007 had a total of 239 Gotland ponies participating. Breeding diplomas was awarded to 46 mares. In 2009, 10 new stallions were approved out of 51 stallions shown (Svenska Russavelsföreningen, 2009).

Conservation strategies

The Swedish board of Agriculture has categorized the Gotland pony as “endangered – maintained” according to the scale used by FAO (Jordbruksverket, 2006). This entitles the breed to subsidies from the European Union. The subsidies are mainly used for information and education (Svenska Russavelsföreningen, 2009). The breed organisation has put together a “plan and guidelines”-document which specifies what a Gotland pony is and how it should be registered. The latest version of the document was approved by the Swedish Board of Agriculture in 2006. However, a special strategy for conserving the breed does not seem to exist.

Gene bank

The gene bank is an initiative from the Swedish Board of Agriculture and its purpose is to act as a backup for genetic material from the Swedish native breeds (Jordbruksverket, 2007). The goal is to have at least 50 animals represented and those should be as little related as possible. The recommendation is that 10 doses of semen are collected from each stallion. The semen collected is not intended for commercial breeding but as a reserve in case of an emergency.

Ponies must have less than 2% Welsh blood to be able to be part of gene bank. A list of stallions that are suggested for leaving semen for conservation in the gene bank has recently been published on the Gotland pony breed society’s homepage.

Population genetics

Inbreeding

Definition of inbreeding

Inbreeding is the result of mating between relatives which leads to a higher level of homozygosity in the population, meaning a decreased level of genetic variability. Genetic variability is a requirement for any kind of improvement through breeding.

Inbreeding is often given as F , the average inbreeding coefficient. The F value is the probability that any allele in an individual is identical by descent with any allele considered in an ancestor (Hartl & Clark, 1997).

Hardy-Weinberg equilibrium

When a population is not subject to any selection, mutation or migration, and gene frequencies are constant from generation to generation it is said to be in Hardy-Weinberg equilibrium (Falconer & Mackay, 1996). In reality some of the things mentioned above always exist. The rule, no selection and random mating, only applies to the genotypes considered. Other genotypes can be both selected and not randomly mated without disturbing the equilibrium for the genotype investigated.

In a population in Hardy-Weinberg equilibrium, rare alleles hardly ever occur in a homozygote state (Griffiths et al. 2005). An allele is considered as rare if it has a frequency of less than 0.005 (Hartl & Clark, 1997). Hardy-Weinberg equilibrium does not apply to sex-linked genes (Griffiths et al, 2005).

The ideal population

In a genetic context, the word “population” most often does not refer to the whole species, but to a geographically isolated group of individuals that can mate with each other (Hartl & Clark, 1997).

In order to simplify calculations of e.g. inbreeding, idealized populations are used. In such a population the following rules apply (Falconer & Mackay, 1996):

- Mating is random
- No selection
- Generations do not overlap
- Number of animals equal for both sexes
- All individuals contribute equally to the gene pool
- The family size follows a Poisson distribution i.e. the variance is identical with the mean

Since no normal population can fulfil these rules, the effective population size is instead calculated.

Effective population size (N_e)

The effective population size is a measure of how many individuals there would be in a population that theoretically is an ideal population with the same rate of genetic drift as the population in question (Hartl & Clark, 1997). Another way of saying the same thing is the number of animals that would be needed to give rise to the same level of inbreeding or variance if the rules for an idealized population were followed (Falconer & Mackay, 1996).

There are several different ways to estimate the effective population sizes. In this study the inbreeding effective size and the variance effective size have been calculated. The first is based on the average change of inbreeding and the last on change in variance of the size in progeny groups for the four selection paths. The estimate of effective population size based on inbreeding rate is the one recommended to use (Woolliams & Toro, 2007), but the variance effective population size may give some additional information on how the breed is managed today and give at least some indications about the future development.

Dramatic increase in inbreeding

A founder effect can arise when a small number of animals start a new sub-population (Falconer & Mackay, 1996). The low number of individuals will cause a large random drift, changes in gene frequencies, in the next generation. This effect will cause the inbreeding to be high since later generations can be traced back to a small group of animals.

Bottlenecks occur when the number of animal have been much reduced due to inopportune conditions. This will also increase the inbreeding in later generations.

Inbreeding depression is due to a high level of harmful homozygous alleles that lower the fitness (Falconer & Mackay, 1996). Traits that can be affected by an inbreeding depression are e.g. litter size and body weight. Inbreeding depression is a drop in mean value for the trait in question relative to the mean for the rest of the population.

Effects of inbreeding

Inbreeding makes it more likely that disease-causing recessive genes will be inherited by the offspring. Highly inbred animals can have lower viability or fertility (Hartl & Clark, 1997). In the Netherlands, van Eldik et al (2006) investigated the semen quality of Shetland ponies with different levels of inbreeding. They found that higher levels of inbreeding resulted in lower percentages of motile and morphologically normal sperm. An effect of inbreeding on percentage motile sperm and sperm morphology could in the study be detected in animals with average inbreeding coefficient between 0.02 and 0.05. Higher levels of inbreeding than this meant larger effect on the semen quality.

Langlois & Blouin (2004) investigated among other things how reproduction in horses was affected by inbreeding. In their data an increase of inbreeding coefficient by 0.01 in the offspring and mare decreased the productivity with 0.005 to 0.010 in the draught and racing breeds. Another study involving the reproduction was made by Sevinga et al (2004), investigating the effect of inbreeding on retained placenta in normal parturition in the Friesian Horse. The average inbreeding coefficient in the population was 0.156 for foals born in 1999, and the rate of inbreeding was 0.019 per generation for the years between 1979 and 2000. A retained placenta after an otherwise normal delivery had a high incidence, 54 %, in the Friesian horse compared to more normally around or below 10 % (Provencher et al 1988). The average inbreeding coefficient for the mares and foals involved were 0.158 and 0.145, respectively. The authors found a positive linear relationship between the inbreeding level of the foal and the occurrence of a retained placenta.

Klemetsdal (1998) found that for the Norwegian trotter, inbreeding lowers the performance in trotting competitions. The higher the level of inbreeding, the more it will affect the racing performance. An increase in inbreeding at low levels will have less effect on the racing performance than the same increase at higher levels of inbreeding.

Generation interval

The generation interval is defined as the average age of the parents when their offspring is born that are to become parents to the next generation (Falconer & Mackay, 2006). The generation interval reflects when the selection of breeding animals is done. The earlier the selection is performed, the shorter the generation interval.

Methods to estimate inbreeding

Genealogical methods

Pedigree analysis

An analysis of inbreeding based on pedigree information measures the probability that an animal has inherited two copies of the same gene from an ancestor. The estimated inbreeding coefficient is given as a value between 0 (not inbred at all) and 1 (completely inbred).

Pedigree completeness index

The more complete a pedigree is, the more likely it is to detect inbreeding. An animal with a high level of completeness in the pedigree may therefore have a higher estimated inbreeding coefficient compared with an animal with less information in the pedigree, even if they are equally inbred. The pedigree completeness index (PEC) is a measure of how complete the pedigree of an individual is. To be able to detect any inbreeding at least parents and one

grandparent are required to be known (MacCluer et al., 1983). This would give a PEC value of 0.24.

Molecular markers

Nowadays molecular methods are used to assign parentage but also to explore the genetic variability in different breeds, e.g. for conservation purposes. Before the era of the DNA, blood groups and blood proteins were used to prove parentage in horses and other mammalian species. DNA has several advantages compared with blood. For example, it can be retrieved from either blood, hair, bones, semen, or other tissues. Samples are also more stable than blood when stored for a long time. There are several different molecular markers at hand, i.e. microsatellites, SNP:s (Single Nucleotide Polymorphisms), RFLP:s (Restriction Fragment Length Polymorphisms), and PCR-RFLP:s (Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms), etc. Today SNP:s, and microsatellites are the most commonly used markers in molecular genetic analysis. The desirable characteristics of a genetic marker is that it is easy to identify, assigned to a specific locus on the chromosome, and that it is polymorphic.

Microsatellites

Microsatellites are repeats of various lengths of nucleotides (Tautz, 1989), they may also be referred to as short tandem repeats (STR's) (Marklund et al, 1994). The number of nucleotides that is repeated can vary, but in horses only dinucleotide repeats have been found (Mikko, 2009, personal message). They also show high levels of polymorphisms (Goldstein & Schlötterer, 1999), and the alleles differ by the varieties in number of repetitions (Brändén, 2003). The high variation is also a reason why microsatellites can be used for parentage testing (Tautz, 1989). The element is most often found in non-coding regions of the genome (Beuzen et al, 2000).

F-statistics

F-statistics is used to measure the reduction of heterozygosity in populations (Hartl & Clark, 1997). The F stands for fixation index and is a way of describing the reduction in heterozygosity if mating is random, relative to any level in a population. F_{IS} is therefore the level of inbreeding in an individual in relation to the inbreeding in the subpopulation to which it belongs. The other two levels are subpopulation in relation to the total population (F_{ST}) and the individual in relation to the total population level (F_{IT}). F_{ST} can only be positive as it is a ratio (Wright, 1965). F_{IT} and F_{IS} can be both positive and negative. The relationship between the different fixations indices is $F_{ST} = \frac{(F_{IT} - F_{IS})}{(1 - F_{IS})}$. F_{IS} cannot be calculated directly from

pedigrees but must be derived from the formula as follows $F_{IS} = \frac{(F_{IT} - F_{ST})}{(1 - F_{ST})}$. If F_{ST} is greater

than F_{IT} the value of F_{IS} will be negative. The reason for negative F_{IS} -values is a higher number of heterozygous animals in relation to the expected number (Marletta et al 2006). From this the conclusion can be drawn that the lower the F_{IS} the more heterozygous individuals there are. A positive value will then mean a larger proportion of homozygous animals. A value of 0.10 or higher indicates inbreeding.

Probability of exclusion

When testing if the animal has the parent as given in the pedigree, the term probability of exclusion (PE) is of importance for the interpretation of the result (Jamieson & Taylor, 1997).

This value gives the probability to detect a false pedigree (Dodds et al, 1996). The PE-values can be calculated in three different ways; PE(1) is the probability of excluding the first parent, PE(2) is the probability of excluding the second parent if the first parent is already known, and PE(3) is the probability of excluding parent pairs. The calculation of PE assumes that the allele frequencies are randomly sampled from the breed, that mating is random for the marker, and that there is no linkage equilibrium between the markers (Bowling et al, 1997).

The exclusion of a male as the father (or for the female as a mother) is more accurate if the other parent is known rather than unknown. In populations with a low number of alleles per locus and low levels of heterozygosity, the accuracy of parentage testing will be lower (Double et al, 1997). Therefore it is important that markers used are highly polymorphic with an even allele frequency. According to Oliveira (unpublished) 17 markers will be needed for the Gotland pony to reach a PE-value of 0.98 for excluding the first parent if the second is unknown. In e.g. the Arabian horse, the Connemara pony, and the Icelandic Horse, the same level of accuracy can be achieved using only 9 markers. To be able to exclude the second parent if the first is already known, 9 markers are sufficient to reach the 0.98 level. The PE values drop as the number of markers and level of inbreeding increases.

Polymorphic information content

The polymorphic information content (PIC) is a measure of the allelic diversity and a measure of how much information each loci can contribute with. A marker is considered polymorphic when it has at least two alleles and the frequency of the most common allele in the population is no more than 0.99 (Shete et al, 2000). A locus is highly informative if the PIC-value is above 0.5 (Botstein et al, 1980). A value between 0.25 and 0.50 means that the loci are moderately informative and PIC-value below 0.25 is slightly informative. Loci with PIC-values close to 1 are desirable.

Similar studies

There have been many other studies investigating different populations with regards to inbreeding using both pedigree analysis and/or microsatellites. When searching for literature the focus has been on studies investigating small populations, since the Gotland pony is a numerical small breed. However other breeds have been included in the literature study for comparison.

Pedigree analysis in other horse breeds

The Greek Skyros horse is a small-sized breed, originating from the island after which it is named, average height at withers is for stallions 1.09 m and for mares 1.07 m (Avdi & Banos, 2008). The breed is considered endangered and less than 200 purebred animals can be found in Greece. The average inbreeding coefficient-value was 0.11 and the annual increase in inbreeding was 0.002 (Avdi & Banos, 2008).

The Spanish Arabian is a sub-population to the Arabian breed (Cervantes et al, 2008). Most of the stallions, 71.2 %, had between 1 and 5 offspring, whereas 1.1 % of the stallions had more than 70 offspring in the studbook. The total number of animals considered as founders were 1626. Pedigree completeness index was relatively high, 92 % for the animals in the most recent generation, but in the 8th generation PEC was only 40 %. No more than 8 generations in the pedigree could be traced even if both parents were known. The average inbreeding coefficient was 0.07. In the whole population, 17.7 % of the horses had an F-value of 0.125 or more.

In Switzerland, there is only one horse breed considered indigenous, the Franches-Montagnes (Poncet et al, 2006). The generation interval varied between 7.6 and 9.2 years. Average inbreeding coefficients for different samples in the study varied from 0.057 and 0.068. The highest average inbreeding coefficient found in the study for stallion and mares were 0.119 and 0.174, respectively. Depending on how rapid the average increase in inbreeding were estimated to be, the inbreeding effective population size was calculated to be 114.5 (increase in F by 0.05 % per year) or 167.8 (increase in F by 0.03 % per year).

Saastamoinen & Mäenpää (2005) published average inbreeding coefficients for some rare breeds originating from Northern Europe. Among the breeds was the Doele horse from Norway, which had an F-value of 0.12. The Doele horse is divided into two types, the more heavy type formerly used for draught work in agriculture and the lighter type used in trotting races. The trotting type is now known as the Norwegian cold-blooded trotter. However, these two types are not allowed to breed with each other.

The Hanoverian warmblood is the largest breed of warmblood horses in the world according to Hamann & Distl (2008). There are approximately 19,000 breeding mares and 420 stallions active in breeding. The PEC values were calculated on five generations in this study. PEC values were high (>0.9) for all categories of animals. The average inbreeding coefficient for all horses was estimated to be 0.00133. The F-value has been rather stable for the breed the last 20 years. The effective population size was 372.34 individuals. The average generation interval was 10 years.

The Trakehner horse is numerically a rather big breed, with about 2400 mares and 248 stallions active in breeding (Teegen et al, 2009). However, the breed has a low number of founding animals since the breed was reconstructed after the Second World War. Effective population size was estimated to be 668.7 animals and generation interval was on average 10 years (Teegen et al, 2009).

Valera et al (2005) investigated the Andalusian breed and found that the average inbreeding coefficient was 0.085. The study concluded that 50 % of the genetic variability could be explained by only 6 individuals. The average generation interval was 10.1 years.

Molecular analysis in other horse breeds

There is one other study performed on the Gotland pony. Cothran (2008) analysed 12 microsatellite markers in a material of 73 animals. Of these 43 animals were from Europe and 30 from America. F_{IS} for the European ponies was 0.025, for the American -0.099 and the combined F_{IS} was 0.025. Observed heterozygosity was 0.648 for all the ponies and the expected level was 0.665. There was a mean number of six alleles for all the Gotland ponies.

Curik et al (2003) analyzed pedigree data and 17 markers in the Lipizzan Horse to determine heterozygosity and inbreeding. They found an average inbreeding coefficient of 0.103, counted on five generations, when using pedigree information from 360 individuals. The mean heterozygosity for an individual was determined to 0.670. The highest number of alleles was found on HTG10, 10 alleles. The lowest number of alleles was 3, on HTG7.

The Portuguese Sorraia Horse is a breed that is considered a critical maintained breed (Luís et al, 2007a). The breed was founded by selecting 10 individuals from the wild in 1937. All pedigrees can be traced back to these individuals. Today less than 200 animals are alive. The

level of average inbreeding coefficient (F) is 0.363. A total of 22 microsatellite markers were used. The mean level of observed heterozygosity for all horses in the study was 0.450. The highest number of alleles was 5; on markers AHT5, ASB2, and VHL20. The lowest number of alleles was 2. The highest observed heterozygosity was 0.705 on marker LEX36. The lowest heterozygosity observed was 0.0088 on marker HMS2.

Luís et al (2007b) compared three Portuguese horse breeds with 30 other breeds to be able to compare level of heterozygosity for future conservation work. In this study the used 12 microsatellite markers. The highest observed heterozygosity was found in the Fell pony breed (0.782) and the lowest in the Friesian breed (0.454). The Sorraia had the lowest number of average alleles, 3.83. The highest number was found in the Caspian horse with 7.75. The Sorraia was the only breed that showed fixed alleles, here found at locus HTG7.

In the Northern parts of Spain there are 4 distinct breeds that are held extensively. Two breeds are of pony-type and two are heavy and mainly used for meat (Solis et al, 2005). A total of 417 animals were analysed for 12 microsatellite markers. The breeds all had high levels of heterozygosity, varying between 0.633 and 0.777. Average number of alleles varied from 5.75 to 8.08.

Ten breeds originating from the Mediterranean area were investigated by Marletta et al (2006). F_{IS} -values ranged from -0.029 in the Spanish Thoroughbred to 0.098 in the Sicilian Indigenous. The expected heterozygosity ranged from 0.690 for the Spanish Thoroughbred to 0.803 for the Sicilian Indigenous. In this study 12 microsatellite markers were used. The least number of alleles found was 6, and the highest was 14.

The Spanish Asturcón is a pony breed that was severely reduced in numbers during the Spanish Civil War (Royo et al, 2007). Since then the breed has been a subject to conservation work. In this study, 1080 individuals with black colour were used for calculating inbreeding. The average inbreeding coefficient was 0.047 estimated from pedigree data. F_{IS} -values were 0.041 for black individuals and 0.013 for bay horses. One breeding farm has had a major influence on the genetic variation. In the beginning 35.6 % of the genetic variation within the breed could be detected to this particular farm. Today it is 50.1 %. An earlier study of this breed by Danner et al (1998) resulted in an F -value of 0.027, whereas the F_{IS} was -0.024. Expected heterozygosity was 0.743 and the observed heterozygosity was 0.712. However, only 451 horses were analyzed compared to 1080 in the more recent study.

In Denmark, Thirstrup et al (2008) performed a genetic analysis on three Danish horse breeds considered indigenous, the Frederiksborg, the Knabstrupper and the Jutland. All three breeds are considered endangered due to their small population number; 980, 781 and 716, respectively. The Frederiksborg and the Jutland studbooks are closed. The Knabstrupper studbook is open but there are discussions about closing the studbook in the future. Inbreeding was calculated on pedigree material for seven generations and inbreeding coefficients were found to be 0.04, 0.03, and 0.06 respectively. The genetic analysis was performed using 12 microsatellite markers. The F_{IS} for all loci are -0.015 for the Frederiksborg, 0.078 for the Knabstrupper, and for the Jutland 0.004. For the Frederiksborg the lowest and highest number of alleles was 3 and 7, respectively. The expected level of heterozygosity was 0.653 and the actual observed was 0.663. The Jutland breed had 2 alleles as the lowest, with 7 as the highest. Expected and observed heterozygosity was 0.613 and 0.611. The Knabstrupper varied between 5 and 9 for number of alleles, and expected and observed heterozygosity was 0.772

and 0.712. The higher values for the Knabstrupper are possibly a result of the open studbook according to the authors of the study.

This study

Material and methods

Pedigree data

Pedigree data was received from the Swedish Horse Breeding Society, and therefore only animals registered in Sweden have been used in this study. The data contained 14973 individuals. Earliest known birth-year in the data was 1900. Information given was database-id, name, birth year, registration-number and name, and id of parents. Data on missing parents was completed to some extent using for example exhibition catalogues and result lists online. A few suspected doublets were found in the pedigree file, where name, birth year and parent id was identical. Some animals that were in the data as parents only were added to the list of individuals.

In total, 14 940 animals were included in the file for inbreeding analysis. In the data, 1184 animals still missed information about the mother and 166 about their father. These animals had a pedigree completeness-value of 0, and their inbreeding coefficients were thus not of interest to present. Among these were the suspected doublets.

Pedigree analysis

Data was sorted and processed using SAS (Statistical Analysis Systems Institute, 1999). For the calculation of inbreeding coefficients (F-values) and pedigree completeness (PEC-values), fortran programmes by Thorvaldur Arnason and Águst Sigurdson were used.

Inbreeding coefficients were calculated according to Wright (1922) using a time-efficient method described by Sigurdsson and Arnason (1995). In this method the pedigree for one animal at the time is traced back and the algorithm of Henderson (1976) and Quaas (1976) is applied.

PEC-values were calculated according to the MacCluer et al (1983) as:

$$PEC_{individual} = \frac{4(C_{father} C_{mother})}{(C_{father} C_{mother})}$$

where

$$C = 1 / d \sum a_i \quad (i=1, 2\dots)$$

a_i = number of known parents in generation i ;

d =number of generations

Calculating effective population sizes

In this study, three measures of effective population sizes were estimated. The inbreeding effective population size (N_{eF}) was calculated based on the rate of inbreeding as $N_{eF} = \frac{1}{2\Delta F}$.

Firstly the following equation was used (Falconer & Mackay, 1996):

$$N_{eF} = \frac{1}{2\Delta F} = \frac{(1 - F_{t-1})}{[2(F_t - F_{t-1})]}$$

where

N_{eF} = inbreeding efficient population size

F_t = average inbreeding coefficient for selected animals in the generation t

F_{t-1} = Average inbreeding coefficient for selected animals in generation t-1

The data was divided in 10-year periods to create “generations”. Selected animals with PEC ≥ 0.8 were used. This gave, however, very varying results for different time periods, as the generations were in fact not fixed but overlapping.

Instead, the inbreeding effective population size was estimated using a log regression of (1-F) on the year of birth (Pérez-Enciso, 1995). The b-value was then equal to $\log(1-\Delta F)$. To get the rate of inbreeding per generation, the resulting ΔF was multiplied with the average generation interval. The effective population size was then calculated as $N_{eF} = \frac{1}{2\Delta F}$. Animals with

PEC ≥ 0.8 , born 1985-2004 were included in this analysis.

In addition, the variance effective population size was calculated using the formula (Hill, 1979):

$$\frac{1}{N_{eV}} = \frac{1}{16N_m L} \left[2 + \sigma_{mm}^2 + 2 \left(\frac{N_m}{N_f} \right) \sigma_{mm,mf} + \left(\frac{N_m}{N_f} \right)^2 \sigma_{mf}^2 \right] + \frac{1}{16N_f L} \left[2 + \sigma_{ff}^2 + 2 \left(\frac{N_f}{N_m} \right) \sigma_{fm,ff} + \left(\frac{N_f}{N_m} \right)^2 \sigma_{fm}^2 \right]$$

where

N_{eV} = the variance effective populations size

L = generation interval

N_m and N_f = the yearly number of new males and females used in breeding

$\sigma_{mm}^2, \sigma_{mf}^2, \sigma_{ff}^2, \sigma_{fm}^2$ = variance of the number of progenies for all selection paths

$\sigma_{ff,fm}, \sigma_{mm,mf}$ = covariance between number of offspring in different paths

Molecular data

Microsatellite marker data was received from the Animal Genetics Laboratory at the Swedish University of Agriculture, comprising a total of 363 registered Gotland ponies. After a comparison with pedigree data, 19 observations were determined to be duplicates. Therefore a total 344 individual had marker information. Table B shows the 16 markers used in the study.

Table B. Microsatellite markers analyzed in this study

Locus	ECA	Reference
VHL20	30	Van Haeringen et al 1994
HTG4	9	Ellegren et al 1992
AHT4	24	Binns et al 1995
HMS7	1	Guérin et al 1994
HTG6	15	Ellegren et al 1992
AHT5	8	Binns et al 1995
HMS6	4	Guérin et al 1994
ASB2	15	Breen et al 1997
HTG10	21	Marklund et al 1994
HTG7	4	Marklund et al 1994
HMS3	9	Guérin et al 1994
HMS2	10	Guérin et al 1994
ASB17	2	Dimoski 2003
ASB23	3	Dimoski 2003
HMS1	15	Guérin et al 1994
CA425	28	Dimoski 2003

There were 241 stallions, 75 mares and 26 geldings in this data. Two animals had unknown sex. Of the 241 stallions, 201 were approved for breeding. The oldest individual with genotype data was born in 1972, whereas the youngest animals were born in 2008. The distribution of animals with available genotype information sorted by birth year can be found in Figure 1.

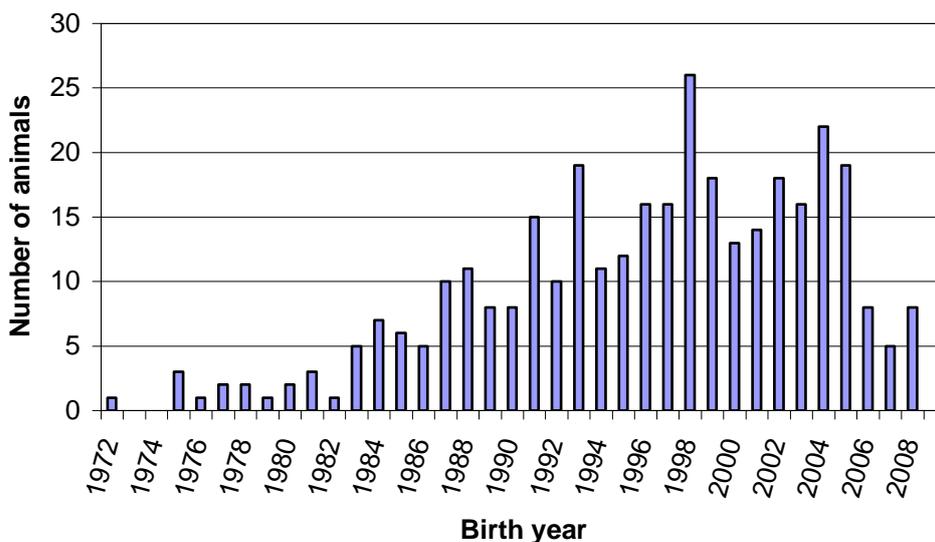


Figure 1. Number of animals with genotype information divided into birth year.

The F_{IS} -values and level of heterozygosity were calculated using the software Genetix 4.05 (Belkhir et al, 1996-2004). The data was divided into different groups before analysis; all animals, breeding stallions, and mares and geldings. Data was also divided into four groups based on birth year. For comparison, animals with DNA profiles were located in the pedigree data and their average inbreeding coefficient (F) was calculated. For the Hardy-Weinberg calculations, Genepop v.4 was used (Raymond & Rousset, 1995). Probability of exclusion and polymorphic information content were analysed for the 344 horses as a single population using Cervus 3.0 (Kalinowski et al, 2007).

Results

Population facts

The number of registered animals every year can be seen in Figure 2. The number of registered animals per year was low until the 1960's.

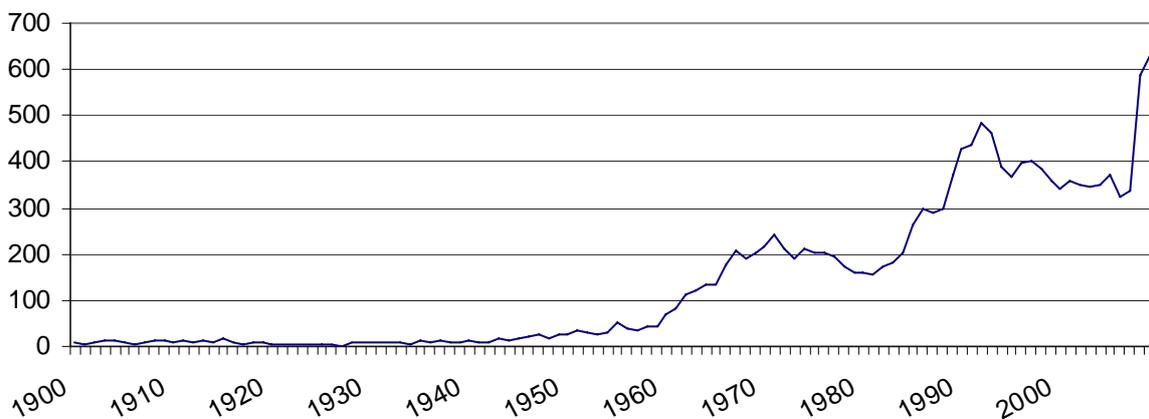


Figure 2. Number of registered ponies per year 1900-2008. The value for 2009 is number of covered mares 2008, and thus the number of horses born 2009 will be reduced when data on actual foalings is in place.

In Figure 3 the distribution of offspring per stallion is shown. The majority of the stallions had 1 to 20 registered offspring. The number of stallions with many progenies was low. The stallion with most progeny, 174, was the stallion Granit 368. Six of the ten stallions with more than 131 offspring had spent between one and three breeding seasons at Lojsta.

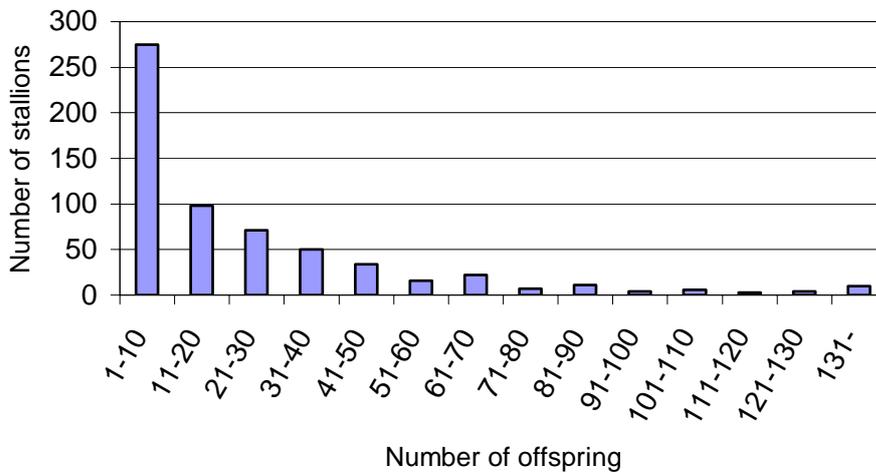


Figure 3. The distribution of the number of offspring per stallion.

Inbreeding and pedigree completeness

The average estimated inbreeding coefficient was 0.11 for all registered animals born in the last “generation”, i.e. 1996-2005. Most of the horses, 98 %, had an F-value between 0 and 19.99. The inbreeding level was high already from the 1930’s. The average inbreeding coefficient for animals born each year can be found in Figure 4. The highest individual average inbreeding coefficient detected in this data was 0.389. The increase in inbreeding per generation was on average 0.5 %, based on differences in average inbreeding coefficients for 10-year periods during the three latest decades. Based on the log-regression analyses of animals born 1985-2004, the increase in inbreeding was 0.75% per generation.

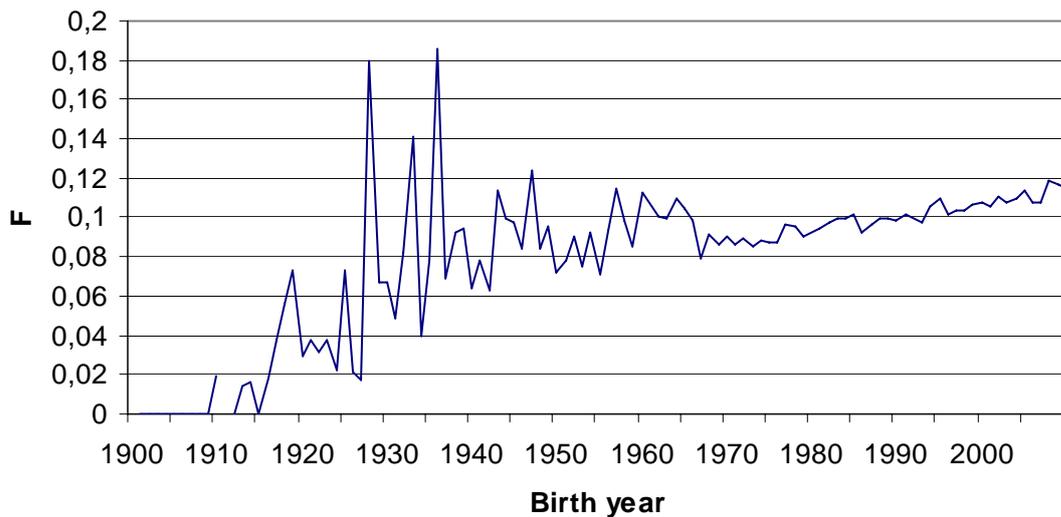


Figure 4. Average inbreeding per birth year for horses used in inbreeding calculations.

Average inbreeding when animals were divided in different PEC-groups is shown in Figure 5. The first animals belonging to the highest PEC-group were born in the 1930’s. The large fluctuations are influenced by the low number of animals born in early years.

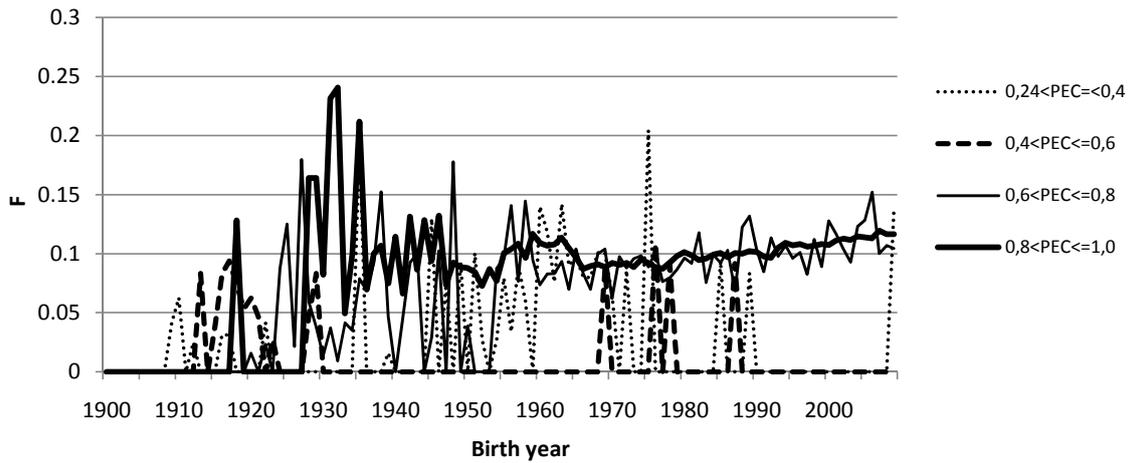


Figure 5. Average inbreeding in different PEC-groups.

The number of animals belonging to the different PEC-groups sorted by birth year can be seen in Figure 6. The majority of horses belong to the highest PEC-group for all birth years since the mid 1930's.

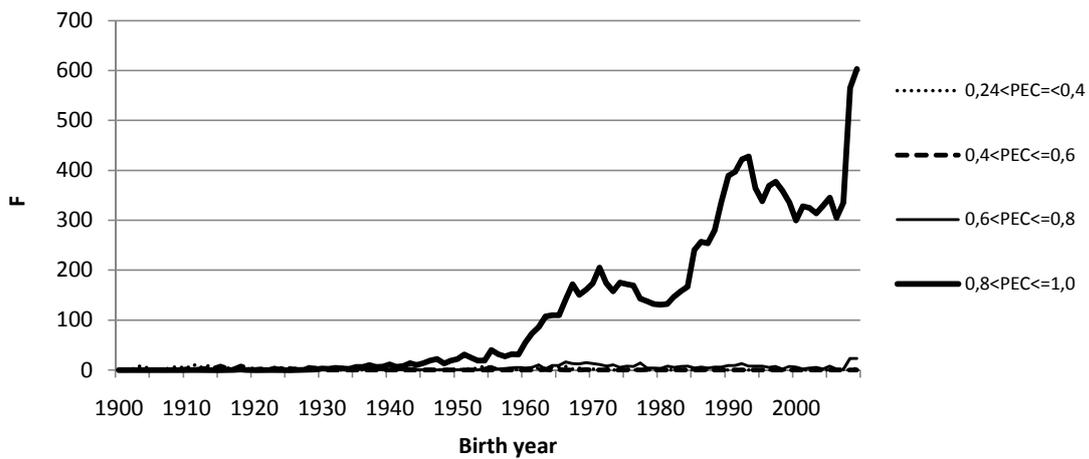


Figure 6. Number of animals belonging to different PEC-groups sorted by birth year. The value for 2009 is number of covered mares 2008, and thus the number of horses born 2009 will be reduced when data on actual foalings is in place.

Generation interval

The average generation interval was 10.4 years. The generation intervals for all four selection paths can be seen in table C.

Table C. Generation intervals

	Generation interval
Sire-son	10.48
Sire-daughter	10.83
Dam-son	9.82
Dam-daughter	10.34

Effective population size

When estimating the inbreeding effective population size as if there were discrete generations it was 88 individuals for the latest generation. How much the estimations of effective population size fluctuated calculated based on the rate of inbreeding between 10-year periods (“generations”) can be seen in Table D. The inbreeding effective population size estimated using log regression was 67 individuals based on the animals selected in the last two generations i.e. 1985 to 2004. The variance effective population size was 235 animals for animals born 1997-2006. Each year on average 10 new stallions were used in breeding and the number of new mares was on average 81.

Table D. The inbreeding effective population size for different “generations” (10-year periods)

Generation	Effective population size
1916-1925	3.90
1926-1935	10.18
1936-1945	-5.83
1946-1955	-37.98
1956-1965	24.19
1966-1975	-49.62
1976-1985	296.74
1986-1995	45.71
1996-2005	88.72

F_{IS} and heterozygosity based on molecular marker information

F_{IS} estimated in the Genetix-programme, was 0.014 for all the animals that had been DNA typed compared to 0.011 for the licensed stallions and 0.015 for mares, geldings and unlicensed stallions. Expected heterozygosity for all animals was 0.64 and the observed was 0.63. For the licensed stallions the expected level of heterozygosity was 0.63 and the observed value was 0.63. The same values for mares, geldings and unlicensed stallions were 0.65 and 0.64, respectively. When animals were divided into different groups based on their birth year the F_{IS} -values came out as can be found in Table E, along with the values for all categories of animals.

Table E. F_{IS} -values, expected heterozygosity (H_E) and observed heterozygosity (H_O) for all categories

Animal category	No of animals	F_{IS}	H_E	H_O
Born 1970-1979	10	0.050	0.62	0.62
Born 1980-1989	58	0.017	0.65	0.65
Born 1990-1999	151	0.016	0.64	0.63
Born 2000-2009	133	0.008	0.63	0.63
All animals	344	0.014	0.64	0.63
Licensed stallions	201	0.011	0.63	0.63
Mare, geldings, unlicensed stallions	141	0.015	0.65	0.64

Expected and observed heterozygosity and the F_{IS} for all loci and all animals with a DNA profile can be found in Table F. One locus, AHT5, stands out distinctively with a F_{IS} at 0.168. There is also a difference between the expected and observed heterozygosity in several loci. ASB2 has rather large heterozygosity and a F_{IS} of 0.088. The average number of alleles per loci is 6.63, with the highest single value being 9 and the lowest 5.

The results for the licensed stallions and the mares, geldings and unlicensed stallions can be found in Table G.

Table F. H_E , H_O , F_{IS} and number of alleles for each locus for all 344 animals investigated in this study

Locus	Expected heterozygosity	Observed heterozygosity	F_{IS}	No of alleles found in this study
VHL20	0.64	0.60	0.060	6
HTG4	0.65	0.66	-0.021	6
AHT4	0.73	0.69	0.045	6
HMS7	0.47	0.49	-0.053	5
HTG6	0.51	0.51	-0.009	5
AHT5	0.53	0.44	0.168	6
HMS6	0.64	0.68	-0.048	6
ASB2	0.75	0.68	0.088	6
HTG10	0.77	0.80	-0.043	9
HTG7	0.52	0.51	0.019	5
HMS3	0.60	0.60	0.015	7
HMS2	0.72	0.70	0.025	9
ASB17	0.77	0.77	0.005	9
ASB23	0.58	0.59	-0.013	8
HMS1	0.58	0.60	-0.036	5
CA425	0.84	0.83	0.014	8

Table G. H_E , H_O , F_{IS} and number of alleles for each locus for stallions compared to mares, geldings and unlicensed stallions investigated in this study

Locus	Licensed stallions			Mares, geldings and unlicensed stallions		
	Expected heterozygosity	Observed heterozygosity	F_{IS}	Expected heterozygosity	Observed heterozygosity	F_{IS}
VHL20	0.63	0.60	0.049	0.64	0.60	0.064
HTG4	0.64	0.67	-0.047	0.65	0.65	0.009
AHT4	0.72	0.71	0.009	0.74	0.66	0.107
HMS7	0.44	0.50	-0.132	0.49	0.47	0.032
HTG6	0.50	0.52	-0.047	0.53	0.51	0.035
AHT5	0.52	0.41	0.217	0.53	0.49	0.087
HMS6	0.62	0.65	-0.045	0.67	0.71	-0.051
ASB2	0.74	0.69	0.078	0.75	0.68	0.102
HTG10	0.76	0.81	-0.066	0.77	0.79	-0.021
HTG7	0.51	0.46	0.084	0.55	0.58	-0.059
HMS3	0.59	0.58	0.017	0.62	0.62	0.004
HMS2	0.72	0.69	0.04	0.71	0.71	0.004
ASB17	0.78	0.74	0.054	0.76	0.80	-0.048
ASB23	0.57	0.59	-0.039	0.59	0.58	0.026
HMS1	0.56	0.59	-0.041	0.60	0.63	-0.038
CA425	0.84	0.83	0.015	0.84	0.85	-0.003

Of the 344 horses with sampled DNA, 331 could be found in the pedigree data. The average inbreeding coefficient for these animals was 0.106. The highest average inbreeding coefficient for an individual was 0.24 and the lowest was 0. In this group, 92 % of the animals had a PEC value above 0.95. The lowest PEC value was 0.73, and that was found for two individuals.

Hardy-Weinberg equilibrium

If the null hypothesis, i.e. random union of gametes, is rejected, the population is not in Hardy-Weinberg equilibrium. A level of significance at 0.05 was used to determine if populations deviated from equilibrium or not. Deviations from Hardy-Weinberg were detected in four loci, AHT5, AHT4, HMS1 and HMS3. On the other hand, when treating the 344 animals all together as one single population, only AHT5 deviated from equilibrium. When animals were divided into generations AHT4 deviated for horses born 1980-1989, HMS3 for individuals born 1990-1999, and finally HMS1 was not in Hardy-Weinberg for horses born 2000-2009. AHT5 deviated for three out of four generations; markers from animals born during the 1980's were in fact in equilibrium.

If horses were divided into licensed stallions and the rest, i.e. mares, geldings and unlicensed stallions, AHT5 and HMS3 were not found to be in equilibrium for the licensed stallions. For the other sexes only HMS1 deviated from Hardy-Weinberg.

Probability of exclusion (PE) and polymorphic information content (PIC)

The PE-values and the PIC-value for all loci can be found in table H. PE(1) ranged from 0.109 to 0.512, whereas PE(2) and PE(3) ranged from 0.187 to 0.681, and from 0.285 to 0.852, respectively. Thus the total PE when combining all 16 markers, is for excluding the first parent 0.992, whereas PE for excluding the second parent and the parent pair was more than 0.999. The PIC values ranged between 0.365 and 0.822 with a combined value of 0.593.

Table H. Probabilities of exclusion and polymorphic information content for all loci

	PE(1)	PE(2)	PE(3)	PIC
VHL20	0.216	0.372	0.536	0.577
HTG4	0.232	0.385	0.557	0.583
AHT4	0.309	0.484	0.663	0.680
HMS7	0.109	0.187	0.285	0.365
HTG6	0.130	0.249	0.383	0.434
AHT5	0.144	0.267	0.409	0.454
HMS6	0.249	0.432	0.633	0.613
ASB2	0.333	0.508	0.685	0.703
HTG10	0.371	0.551	0.737	0.730
HTG7	0.139	0.281	0.433	0.468
HMS3	0.205	0.378	0.565	0.564
HMS2	0.296	0.466	0.643	0.665
ASB17	0.395	0.576	0.767	0.743
ASB23	0.202	0.386	0.595	0.557
HMS1	0.183	0.344	0.520	0.531
CA425	0.512	0.681	0.852	0.822
Total (all markers)	0.992	0.999	0.999	0.593

Discussion

Data

Completeness of data

The pedigree data was rather complete indicated by the fact that animals belonging to the highest PEC-group (0.8-1) was born as early as in the 1930's. However, over one thousand animals had missing parental information. Some of the missing pedigree information could be found in the studbooks but since the animals did not exist in the dataset from SH, the information could not be used to complete the missing information. There were also some doublets found in the data.

If one or both parents to an animal were not registered in Sweden the information unfortunately came out as missing. Hopefully the database at SH used to generate the data material for this study will incorporate this sort of data when the animal is not really missing the pedigree information. The same applies for the animals found in the studbook but not present in the database.

Population facts

Number of registered animals

The number of animals registered per year was very low for some years in the beginning of the 20th century as can be seen in Figure A. One reason could have been that animals were not allowed to be evaluated on the mainland of Sweden. Another reason, and perhaps the most likely, is that there were very few animals at all in the breed. From 1961 the ponies have also

been evaluated in the mainland and since then the number of registered animals has steadily increased with a peak in the middle of the 1990's with almost 500 registered animals. Another reason for the increase in numbers could be that horseback riding and trotting became popular among a wider group of people and therefore there was a market for ponies of this size.

Number of offspring per stallion

The majority of stallions used in breeding had 10 or less registered offspring. Among the 10 stallions with more than 131 offspring, 6 had been the stallion used on Lojsta one or more seasons. For keeping the increase in inbreeding at a low level, it is desirable that the variance in progeny group size is low, with rather small differences between stallions.

Generation interval

The generation intervals estimated in this study are reasonable compared with literature. The average generation interval for the Gotland ponies was 10.4 years. This is similar to generation intervals given in similar studies in other horse breeds e.g. Valera et al (2005) and Hamann & Distl (2008). An example of a breed with shorter generation interval is the Franches-Montagne, which had a generation interval between 7.6 and 9.2 years (Poncet et al, 2006).

Effective population sizes

The inbreeding effective population size varied a lot between time periods when it was based on the difference in average inbreeding coefficient in different 10-year time periods ("generations"). If the inbreeding was decreasing or increasing dramatically, e.g. as a result of few registered animals in one "generation", it would show in the effective population size. If the inbreeding decrease, it results in negative population sizes (Gutiérrez et al, 2008), as it did in some of the generations for the Gotland pony. The big fluctuation was the reason for instead using log regression. The inbreeding effective populations sizes of 67 animals found here indicate that the breed is not in a too bad condition. The variance effective population size was based on the variation in offspring group sizes. The resulting 235 animals is an indication of how the breed is managed.

Genealogical results

High inbreeding from the beginning

The reason why the inbreeding was particularly high some years in the early decades of the 1900's is the fact that only a few animals were registered. If the few animals that year each had a high inbreeding coefficient it will reflect in the average value. The average inbreeding for all animals was around 0.02 and 0.04 between 1900 until 1930, when it rose above 0.06. After that it has not dropped below 0.06 again. The level of inbreeding has been rather stable around 0.1 - 0.12 in the last decades. The increase in inbreeding is not very dramatic; it is under the critical limit of 1% per generation, but above the recommended level of 0.5 %.

There is a possibility that there has been a bottleneck before 1900. In that case it will not have been detected in this data. Since the level of inbreeding is high in individuals born early in the 1900's it is plausible that a bottleneck in fact did occur. Another bottleneck may also explain the increase in inbreeding coefficient that occurred after the 1930's.

Negative effects of a high average inbreeding coefficient within the breed

High levels of inbreeding can reduce the fitness in a breed. Fertility is often one of the characteristics affected. Compared with other pony breeds such as the Welsh pony, the New Forest and the Connemara, the Gotland pony is comparable in the foaling percent i.e. the number of born foals in relation to the number of covered mares the previous year.

However, the foaling percentage may not be a good measure of a stallion's performance. This measure does not take into account the female fertility and the fact that much can happen after the mare has left the stallion. A stallion's performance can also be affected by e.g. disease and management. Therefore it could be of interest to investigate if the high level of inbreeding in this breed has led to any effects on the fertility. Perhaps one could see if stallions and mares that are less related to each other tend to have better chance of a pregnancy than if the stallion and mare are closer related. Also the capacity of a mare to keep the foetus and give birth to a living foal can be of interest. Something else related to foaling is the delivery itself. Is there a higher level of difficult births or higher number of foals that are incorrectly positioned during the delivery? Looking at the foaling percentage alone is perhaps not the best way of determining if there is reduced fertility within the breed.

Another thing affected by inbreeding is performance. Since the Gotland pony is dominating the trotting races for its size category, an analysis of competition results would be of interest. A study performed on the Norwegian trotter showed that inbreeding lower the level of performance. Therefore the hypothesis would be that the trotting performance in the Gotland pony would be negatively affected as well. Exhibition/conformation show results could also be analyzed. Since inbreeding gives more similar animals, perhaps inbred animals would get a higher award. It could also be of interest to see if there are different families used in trotting compared to showing in breeding/conformation shows, and if there are any differences in inbreeding between them.

How to keep the rate of inbreeding steady, and how to decrease it

One important task for the breeders of the Gotland pony is to keep the rate of inbreeding steady and low. Since the breed has a closed studbook this will have to be done with the animals that are registered within the studbook. The use of welsh stallions in the 1950's and 1960's was debated at the time and such an outcross strategy may not be likely to happen again, unless it is absolutely needed. One way of keeping the genetic variation is to maintain as many stallion and mare lines as possible. This is done today at stallion approvals. A stallion with a rare pedigree may be approved as a breeding stallion without reaching the limit of 40 points.

Breeders can also actively use breeding animals that are as little related as possible. If for example a mare owner has found a number of stallions that will suit their mare, the one that is less related to the mare should be considered before the others.

If sometime in the future the decision is made to use BLUP (Best Linear Unbiased Prediction) as a help in breeding, consideration should be taken to the effect this may have on the level of inbreeding. There is software available, such as Gencont or EVA, that can aid in deciding how many offspring different stallions should get to keep the average kinship in the population low (using optimum contribution selection).

The inbreeding situation in the Gotland breed does not motivate any extraordinary actions today. If, however, inbreeding levels would rise alarmingly in the future, and the problems

cannot be managed using the breeding material within the studbook, one possible solution would be to once again bring in some breeding animals from another breed. The breed chosen should in that case be similar to the Gotland pony when it comes to how it looks and is built, as well as the history of the breed. It is also preferred if the breed is somewhat related to the Gotland pony in a molecular aspect. Cothran (2008) found that the Connemara, the Exmoor and the Shetland breeds show relationship with the Gotland pony. Of these three breeds the Exmoor is the closest to the Gotland pony when it comes to size and conformation and might therefore be a good choice.

There are small populations of Gotland ponies in the other Nordic countries as well as in America. Some of these animals might be less related to the population in Sweden and may therefore be of great value in breeding.

Molecular results

DNA-animals already selected

To be able to detect 95 % of the genetic variety present in a breed only around 40 unrelated animals are needed if microsatellites are used (Mikko, S. Personal message). Therefore 344 animals should be sufficient enough. And since all animals were investigated for 16 markers and there are at least two alleles for each locus, the data is theoretically approximately 11 000 data points. However, because most of the DNA-samples from the Animal Genetic Laboratory are from licensed stallions, the samples are not likely representative of the whole population. Therefore the animals were divided into three groups when investigated. Still, the F_{IS} was approximately 0.01, and this is tenfold less than when calculated from pedigree information, for all groups except for the animals born 1970-1979, but they were on the other hand only 10 individuals which most likely affected the result.

F_{IS} for the different loci

There were some loci that showed higher F_{IS} -values than others. When considering all 344 animals as one population, the loci VHL20, AHT5, and ASB2 all have F_{IS} -values over 0.050. AHT5 have the highest value of 0.168. These are all loci that normally have a high level of probability of exclusion that follows a high level of variation and thus a high PIC value. When divided into licensed stallions and others, the loci with highest F_{IS} were different between the two groups. For the licensed stallions the loci with the highest values were VHL20, AHT5, ASB2, HTG7, and ASB17. The F_{IS} were 0.049, 0.217, 0.078, 0.084, and 0.054, respectively.

For the other group e.g. mares, geldings and unlicensed stallions the loci with higher F_{IS} -values than 0.050 were VHL20, AHT4, AHT5, and ASB2. The F_{IS} -values for these loci were 0.064, 0.107, 0.087, and 0.102, respectively. Number of loci with F_{IS} over 0.050 was greater for the licensed stallions compared with mares, geldings and unlicensed stallions with marker information. This indicates that the stallions belong to a selected subpopulation with somewhat higher degree of inbreeding. When looking at e.g. AHT5, the F_{IS} for the licensed stallions were 0.217 compared to 0.087 for the other group. This again indicates that the licensed stallions are a more homogenous group at DNA-level.

Why negative F_{IS} -values?

The reason for negative F_{IS} -values is a higher number of heterozygous animals in relation to the expected number (Marletta et al 2006). The fact that a number of loci show negative F_{IS} indicates that these loci still have an amount of variation within themselves. This is positive

news as it indicates that despite a high level of inbreeding estimated from pedigrees, there is variation to work with in future breeding.

High F_{IS} -values are more easily detected in markers with high probability of exclusion

The markers with a 0.05 or higher F_{IS} -value in all the groups were VHL20, AHT5, and ASB2. These are all markers with a high probability of exclusion. A high probability means that there are many alleles, a high level of information in every marker and the frequencies of alleles are evenly distributed. If there is a decrease in number of alleles in a marker with many alleles, it may be detected more easily than in a marker with few alleles.

Since the Gotland pony needs so many markers for a good result in e.g. parentage testing, perhaps inbreeding is easier detected in markers that are highly variable. Some markers may then be of less importance when trying to estimate the level of inbreeding because they already have so little information.

Number of alleles per locus

The lowest number of alleles detected in this study was 5 and the highest was 9. This shows that none of the studied alleles have become fixed in the population. The average number of alleles per loci was 6.63 in this study. This is almost the same as Cothran (2008) found in his study. The fact that some loci had a high F_{IS} -value and were not in Hardy-Weinberg equilibrium mean that in the future there is a risk of alleles becoming fixed.

Hardy-Weinberg

The fact that some loci show a deviation from Hardy-Weinberg equilibrium, indicate that some sort of selection is taking place for the loci concerned. The loci that deviated from Hardy-Weinberg across the different groups were AHT5, AHT4, HMS1, and HMS3. All these loci are located on different chromosomes so the position on the chromosome is not the explanation. The explanation might be that some traits are associated to these loci and therefore a selection has taken place. What these traits are can only be speculated upon. Perhaps it has something to do with fitness, or it can be a performance trait.

Another locus that is close to deviation from Hardy-Weinberg equilibrium, if a significance level of 0.05 is considered, is HMS7. The p-value for this locus when all 344 horses are considered was 0.0503 and for the licensed stallions the same value is 0.0521. This locus is situated on chromosome 1, which is not the identical to some of the loci that actually did deviate from equilibrium.

Probability of exclusion and polymorphic information content

Interestingly, for all three PE values, i.e. PE(1), PE(2) and PE(3) the least informative marker was HMS7, and the most informative was CA425, that also correlated with their PIC-values in the Gotland pony breed. This implies that for this breed, CA425 is an important marker to include in parentage controls. Also, since PE(1) stands out with a lower probability of exclusion than PE(2) and PE(3), it is highly recommended to include both parents in the parentage control of Gotland ponies, as well as a sufficient number of markers.

PIC-values above 0.5 existed for 12 of the 16 loci used in this study. The highest value was 0.822 for the CA425 locus, supporting that this marker has an important and informative role in population genetic analysis of this breed. The remaining four, loci HMS7, HTG6, AHT5, and HTG7, had PIC-values varying between 0.365 and 0.468, i.e. moderately informative.

This implies that even HMS7, the marker with the lowest PE- and PIC-values, is still useful in these calculations. In other species, AHT5 normally have higher PIC-values. The lower PIC-value for this marker in the Gotland pony may reflect that traces of inbreeding is more clearly detected in markers that normally exhibit high PIC-values. Thus, if the analyzed markers have too low PIC-values, the inbreeding level may not be detected with high F_{IS} values as expected.

These results mean that for the Gotland pony 12 markers were highly informative and the other four are moderately informative. Because of this more markers will be needed in e.g. parentage testing to reach a reliable result, compared to other breeds which have more information in each marker.

Correlation between genealogical and molecular information

Is there a correlation between genealogical and molecular information

In a study performed on dogs by Leroy et al (2009) only a few non-zero correlations were found between genealogic and molecular parameters. On average they tested 25 dogs per breed. Correlations between genealogical and molecular parameters were estimated. The correlation between F and F_{IS} were determined to be -0.21. Leroy et al also proposed an explanation for the difference between F and F_{IS} , that genealogical information is a way of showing more recent effects, while molecular data shows a more accumulated effect.

Baumung & Sölkner (2003) used simulations to study if pedigree or marker information was more effective in finding autozygotes i.e. the real proportion of loci with alleles identical by descent. They found that when breeding is random, inbreeding coefficients based on pedigree is more reliable than information from markers in finding autozygous animals. When breeding is not random there is a higher need for correct pedigree information. The correlations between marker inbreeding coefficients and the true autozygosity drop a little when concerning non-random matings compared to random matings. Lack of information about true level of allele frequencies cannot be compensated by increasing the number of loci investigated. Baumung & Sölkner (2003) came to the conclusion that pedigrees with little information identified most of the autozygous animals. To reach the same result using marker information, over 100 marker loci had to be analyzed. Since this study on Gotland ponies used pedigrees with much information and a high level of completeness it is plausible that most of the autozygous animals were found.

Balloux et al (2004) investigated if heterozygosity was correlated with inbreeding estimated from genealogical information. They found that the correlation between heterozygosity and inbreeding was low, independent on the number of molecular markers. This was however not the case if the population size was small, had strong substructures within the population, or extreme breeding systems leading to a high number of matings between close relatives. So according to this heterozygosity is not a good measure of inbreeding in a population. However, one could question if the Gotland pony could fit with the conditions of a small population size and high number of matings between relatives. If that was the case then heterozygosity can be a good measure of inbreeding in the Gotland breed.

The fact that the breed has rather high levels of heterozygosity could indicate that animals that are heterozygous are favored by selection, as Głazewska & Gralak (2006) showed for the Polish Arab horse.

Conclusions

Based on genealogical methods the breed is relatively inbred with an average inbreeding coefficient of 11%. However, the increase in inbreeding per generation was 0.75% and that is a more important measure. Inbreeding effective population size was 67 ponies. The variance effective inbreeding population size was 235 horses. The level of inbreeding within the population will continue to rise but increase in inbreeding per generation should not be allowed to be higher. However, the effective population sizes indicate that there is still breeding material to work with. Breeders should take the level of inbreeding in mind when choosing breeding stock, e.g. if there are several equally good alternatives for a stallion, the one least related to the mare in question should be chosen.

F_{IS} for the whole population estimated from molecular data was 0.014. The highest F_{IS} for a single locus was for AHT5 with 0.168. Observed heterozygosity is 0.63. One locus, AHT5, was not in Hardy-Weinberg equilibrium. Inbreeding calculated from molecular data is about ten times less than that based on pedigree information. Some loci show moderate levels of inbreeding but there is still much variation to work with in future breeding.

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