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SNP-baserad bevarandegenetik för den södra svenska brunbjörn (*Ursus arctos*) populationen

Joanna Fahlén

Examensarbete i ämnet biologi
Department of Wildlife, Fish, and Environmental studies
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Joanna Fahlén

Supervisor:	Göran Spong, Dept. of Wildlife, Fish, and Environmental Studies
Assistant supervisor:	Anita Norman, Dept. of Wildlife, Fish, and Environmental Studies
Examiner:	Carl-Gustaf Thulin, Dept. of Wildlife, Fish, and Environmental Studies

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Abstract

Species with small population sizes needs to be managed in order to prevent extinction in the long-term. Genetic monitoring of wild populations over time is important since it enables for management strategies that takes the genetic status into consideration. Non-invasive sampling techniques are useful for rare and elusive species since the organic material containing DNA is collected through, for example, hair, saliva or feces without handling or even disturbing the animal. Single nucleotide polymorphisms (SNPs) are a molecular marker that amplifies well when using low quality or degraded DNA, as often is the case with non-invasive samples. The aim of this thesis is to genetically characterize the previously bottlenecked southern Swedish brown bear population within the counties of Dalarna and Gävleborg. Non-invasively sampled DNA from 434 bears was genotyped on a recently developed SNP-panel with 96 loci. The analyses of genetic diversity resulted in an observed heterozygosity that was close to the heterozygosity expected under ideal conditions (H_o 0.45, H_e 0.49). There were no indications of inbreeding (mean F_{IS} -0.0014). Seven males from a different population were identified and these males are likely first generation immigrants from the northern population, which indicates gene flow. No population structure within the southern population was found, possibly due to the high mobility among males. The effective population size (N_e) was 74.4 and the N_e/N_C ratio 0.094. According to recommendations of N_e, which are set to prevent inbreeding and to ensure long-term viability, the N_e of the southern population could increase. Further gene flow from the northern population will likely enable this scenario.

Introduction
A background to conservation genetics

The human world is expanding and the competition for use of a finite land base intensifies (Shaffer 1981). The ever ongoing exploitation of natural environments causes fragmentation and loss of suitable habitats for a wide range of species, thereby putting their future survival at risk. The International Union for Conservation of Nature (IUCN) details the global conservation status of species and has put 1 199 out of 5 513 described mammals in either of the Red List categories Critically Endangered (CR), Endangered (EN) or Vulnerable (VU) (IUCN 2014). Conservation is about preventing species from going extinct and to help them persist in the future. The number of individuals of a certain species is directly proportional to long-term viability, where a large population is less likely to become inbred and thus has greater survival chances than a small one (Shaffer 1981). However, conservation efforts that aim to sustain a certain species in the long-term require knowledge not only about the number of individuals in a population, but also about the genetic characteristics. Genetic monitoring of wild populations over time enables for management strategies that takes the genetic status and health into consideration. Conservation genetics is an area of study that is based on the information that can be found in the DNA and questions regarding, for example, genetic diversity, inbreeding, gene flow, population structure and effective population size can be addressed through the use of certain molecular markers.

Genetic diversity and inbreeding

The genetic diversity has substantial effects on the evolutionary potential and long-term viability of a population (Waits 1999) and is recognized by the IUCN as one of three levels of biodiversity that deserves conservation. The other two levels concerns species and ecosystems (McNeely *et al*. 1990). Genetic diversity is created and maintained through either mutation or gene flow. The latter is important due to the recombination of homologous chromosomes during meiosis, which leads to novel combinations of genes along a chromosome (Freeland *et al.* 2011). Genetic diversity is described as the number of alleles or the proportion of polymorphic loci at a certain gene location. Populations with high genetic diversity have good evolutionary potential and thereby great prospects to adapt to unexpected changes in abiotic and biotic factors such as temperature or different diseases (Reed and Frankham 2003). In contrast, populations with low genetic diversity have limited evolutionary potential and might not be able to adapt to changing factors since necessary alleles have been lost through genetic drift (Slatkin 1987). Low genetic diversity can be a consequence of inbreeding, i.e. mating among close relatives, which results in a population that has an excess of homozygotic genotypes with alleles that are identical by descent. This can lead to an overall lowered fitness, so called ‘inbreeding depression’, and an inbred population may consequently suffer from an increased risk of extinction (Reed and Frankham 2003).

Population structure and gene flow

Most species show some geographic variation in gene frequencies, i.e. population structure, and the extent of the differentiation among local populations is determined by diverging factors such as mutation (adds variation), genetic drift (removes variation) and natural selection (either removes or retains variation) (Reed and Frankham 2003; Frankham *et al*. 2014). Gene flow, on the contrary, reduces the genetic differentiation between local populations and can either promote evolution by the spreading of new genes or, alternatively, constrain evolution by preventing local adaptation (Slatkin 1987). Assessing gene flow by measuring the direct number of migrants is important in order to gain insight into the connectivity between two or more populations as it affects the levels of genetic diversity,

inbreeding and genetic drift. One reproductive migrant per generation increases the genetic diversity and fitness within a small and inbred population, while at the same time allowing for divergence in allele frequencies between subpopulations (Mills and Allendorf 1996). When the effective population size (N_e) is much lower than the census population size (N_C), however, more than one migrant per generation is needed in order to increase the effective population size, but more than ten migrants per generation could potentially cause uniformity in allele frequencies between subpopulations (Mills and Allendorf 1996).

Effective population size and minimum viable population sizes

The effective population size is a measure of the genetic size of a population relative to that of an 'ideal' population. An ideal population is randomly mating, has an equal sex ratio and non-overlapping generations (Wright 1931). N_e is further defined as "*the size of an ideal population that would result in the same level of inbreeding or genetic drift as that of the population under study*" (Jamieson and Allendorf 2012, p.578). In reality there are no ideal populations, but the effective population size is nevertheless an informative and important parameter within conservation and management because of its relationship to inbreeding accumulation and loss of genetic diversity (Nomura 2009; Robinson and Moyer 2012). Accurate estimations of N_e are therefore important in order to properly manage population sizes so that low genetic diversity is prevented and long-term viability is ensured. Old recommendations (Franklin 1980; Soule 1980) suggest that a population with a N_e of ≥ 50 individuals is needed to avoid inbreeding depression in the short term (about five generations), while a long term N_e of ≥ 500 individuals is required to retain sufficient quantitative genetic variation and ensure long-term viability. This is also known as the"50/500 rule-of-thumb". However, these recommendations have recently been revised by Frankham *et al*. (2014), who suggest a new short term N_e of 100 in order to keep the inbreeding rate at less than 10% during the following five generations, and a new long term N_e of 1 000 in order to maintain evolutionary potential in the long perspective. Furthermore, if the N_e/N_C ratio has been estimated, it is possible to calculate the theoretical population size that is required for fulfillment of both the short-term minimum viable population (MVP) criterion of $N_e \geq 50$ or 100, and the long-term MVP criterion of $N_e \geq 500$ or 1 000 (Nilsson 2013). There are four common methods to calculate N_e, and these include: (i) the temporal method (Nei and Tajima 1981; Pollak 1983), (ii) the linkage disequilibrium (LD) method (Hill 1981), (iii) the heterozygote-excess method (Pudovkin *et al*. 1996), and (iv) the allele sharing method (Nomura 2008). The temporal method requires at least two samples from different cohorts (age groups), while the other three methods are single-sample estimators that enable estimations of N_e through samples that have been collected from one generation or one field season (Nomura 2009). Genetic estimations of contemporary N_e through the use of single sample methods is nowadays the main choice for researchers in studies concerning endangered animals with long generation times (Robinson and Moyer 2012). Linkage disequilibrium can be described as "*the non-random assortment of alleles in a population at two or more loci into gametes*" (Hedrick 2005) and the basis of the LD method is that linkage disequilibria decay at an exponential rate, which is determined by the amount of recombination during meiosis. The balance is maintained through random creations of new disequilibria in each generation (Waples 2005). The LD method is suitable for single-sample data since it does not require detailed information on life-history parameters or the age of individuals, and it is the method that provides the most accurate estimate of N_e for species with overlapping generations (Robinson and Moyer 2012). Certain assumptions associated with the LD method are that the study population consists of diploid, random mating individuals, including loci that are physically unlinked and in Hardy-Weinberg equilibrium (HWE) (Hills 1981; Waples and England 2011).

Brown bear phylogeography

For about 10 000 years during the Pleistocene and the last glacial maximum (ca. 23 000 - 18 000 BP) brown bears (*Ursus arctos*) were restricted to three main unglaciated refugia in the Iberian, Italian and Balkan Peninsulas (Taberlet and Bouvet 1994; Hewitt 1996; Sommer and Benecke 2005; Valdiosera *et al.* 2007). Recolonization from the Iberian refugium into mainland Europe occurred directly following the retreat of the ice caps, while the Alps acted as a migratory barrier for bears in Italian and Balkan refugia, hence delaying their northward expansion until most of Europe had been recolonized by other populations (Hewitt 1999; Sommer and Benecke 2005). It has been proposed that crossing of the land bridge between Denmark and Sweden happened during the Bølling-Allerød interstadial (ca. 13 000 - 11 000 ^{14}C yr. BP), which was a warm period at the end of the Pleistocene. Brown bears then survived in southern Sweden during the cold epoch of youngest Dryas (ca. 11 000-10 000 ^{14}C yr. BP), with no possibility to migrate in any direction due to the flooded Öresund channel in the south and intact ice caps in the north (Mangerud *et al.* 1974; Björck 1995; Sommer and Benecke 2005; Hoek 2008). The northern expansion and recolonization started once more when warm ocean currents from the Atlantic induced melting of the ice, and around 6 000 years BP the vegetation became similar to what it is at the present (Hewitt 1996).

Two distinct mtDNA lineages in Sweden

Molecular studies have shown that modern European brown bears are divided into two main mitochondrial DNA (mtDNA) lineages dependent on ancestral refugium: one is referred to as the eastern lineage and is comprised of populations from northern Scandinavia, Russia and Romania, while the other is referred to as the western lineage which is divided into two clades, (i) the Iberian clade (populations in southern Sweden, Norway, Pyrenean- and Cantabrian Mountains) and (ii) the Balkan clade (populations in Abruzzo, Trentino, Slovenia, Bosnia, Croatia, Greece and Bulgaria) (Taberlet and Bouvet 1994). These two mtDNA lineages differ from each other by a mean pairwise genetic distance of 7.13% and the contact zone is located in the middle parts of Sweden (Fig. 1a) (Taberlet and Bouvet 1994; Taberlet *et al*. 1995). Bears north of the contact zone belong to the eastern lineage and migrated into northern Scandinavia via Finland and Russia from a refugium in the Carpathian Mountains, while bears south of the contact zone belong to the western lineage and the Iberian clade (Taberlet and Bouvet 1994). A study by Saarma *et al*. (2007) suggests that the most recent common ancestor of all European brown bears lived approximately 175 000 years BP. Henceforth in this text, bears in Sweden of the eastern lineage are referred to as the northern population, while bears of the western lineage are referred to as the southern population.

Historical and current status in Sweden

Brown bears in Europe were extensively hunted from the 17th well into the 20th century, mainly due to their valuable meat and fur, but also to prevent predation on livestock (Servheen *et al*. 1999). In 1647, Sweden introduced national killing bounties and offered quite low rewards. Eradication campaigns in most parts of Europe during the 19th and 20th centuries led to an increase of the Swedish bounties in 1864 so that the value of a dead bear became equivalent to the value of a cow (Lönnberg 1929), which further boosted the hunting. The eradication campaigns were successful in most parts of Western Europe, and only small remnants of a former widespread species were all that remained when the bounties were removed (Swenson *et al*. 1995; Swenson *et al*. 2011a). Sweden removed the bounties in 1893, followed by a hunting ban on Crown land in 1913. In order to prevent extinction, all brown bears in Sweden were declared Crown property in 1927, which removed the last economic incentives for killing bears (Lönnberg 1929). The population size in Sweden was at its lowest point in 1930 with an estimated number of only 130 individuals distributed over four female

core areas (Swenson 1994). The number within the southern population was as low as approximately 50 bears. However, the Swedish population increased in numbers and hunting was allowed once again when the protection by law was withdrawn in 1945 (Swenson *et al*. 1995). The bottleneck in the 1930s likely resulted in lowered levels of genetic diversity, but the population recovered and the levels were high once again at the beginning of the 21st century (Waits *et al*. 2000; Tallmon *et al*. 2004). The recovery in genetic diversity has been hypothesized to be a result of male-mediated gene flow between the core areas. Based on mtDNA studies, Taberlet *et al*. (1995) found four males on the 'wrong' sides of the contact zone; two on the southern side and two on the northern side. Norman *et al*. (2013) found two males and Waits *et al*. (2000) found four males within the southern population that belonged to the northern population. These findings provide support to the theory about male-mediated gene flow between the northern and the southern population. The population size peaked in 2008 with 3 300 bears (Kindberg *et al*. 2011), followed by a significant decline of 3.2% per year, leading to the current number of about 2 800 bears (Fig. 1b) (Kindberg and Swenson 2014). The northern population seems to decline the most and one explanation is that this might be due to poaching (Servheen 1999; Swenson *et al*. 2011b).

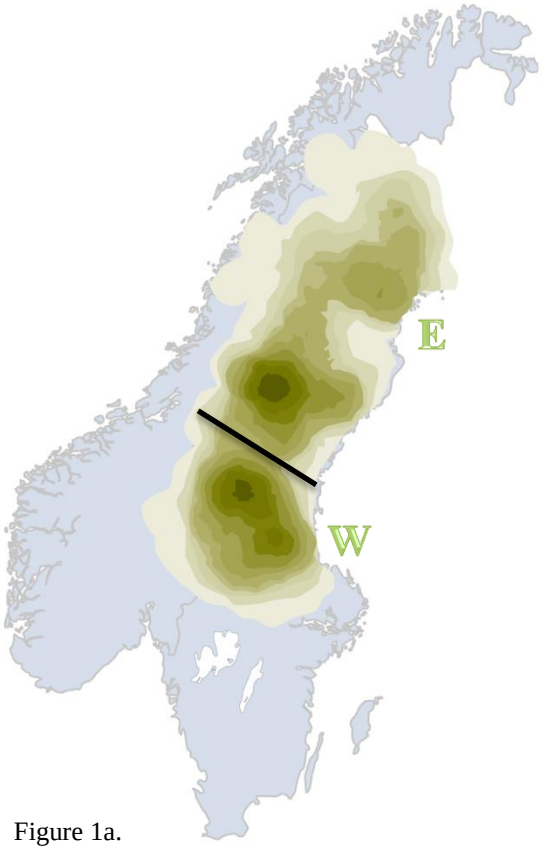

Figure 1a.

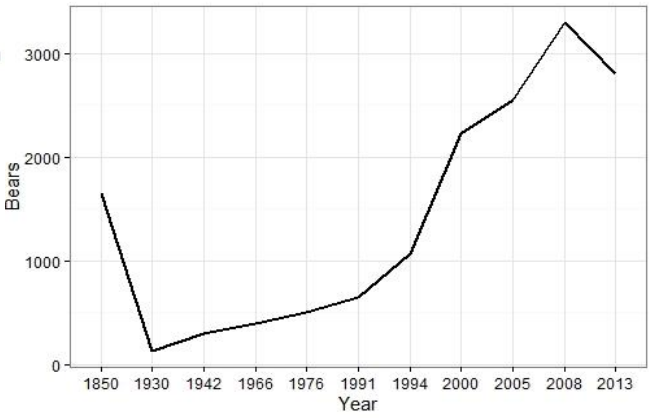

Figure 1b.

Figure 1a. The map shows the distribution and abundance of brown bears in Sweden based upon fecal DNA, sampled between the years 2004-2012. Dark areas indicate a higher abundance of bears than light areas. The letters 'W' and 'E' stands for the western and the eastern lineage, respectively (Taberlet *et al.* 1994). The black line is the contact zone, which demarcates the two populations. This picture is modified from Kindberg and Swenson (2014) and the placing of the contact zone is from Taberlet *et al.* (1995). Figure 1b. Fluctuations in population size from 1850 to 2013.

Genetic sampling and SNPs

Sampling of organic material containing DNA is an essential step that precedes all genetic studies. Common forms of DNA-sampling from wild animals have involved clipping of skin or hair, and the extraction of blood or body fluids (Sherwin 1991). These methods are referred to as invasive sampling since they are associated with handling, which might be stressful for the animal. Although, handling the animal might be necessary in some studies since it enables the attachment of different tracking devices such as GPS-collars and the collection of individual data, for example, weight, age or body measures. However, handling can be both difficult and unsuitable when it comes to rare, elusive and perhaps endangered animals. In these cases, collection of genetic data is best done through non-invasive sampling, which is the sampling of organic and often partially degraded material such as feces, hair or saliva without handling or even disturbing the animal (Waits 1999; Bellemain *et al*. 2005).

SNPs as molecular marker

Technical advances within next-generation sequencing techniques over the past ten years have turned single nucleotide polymorphisms (SNPs) into a popular molecular marker. SNPs are a fundamental type of genetic variation with high genomic resolution as they occur frequently throughout the genome within both coding and non-coding regions (Morin *et al*. 2004). Essentially, SNPs are single-nucleotide differences between members of the same species, and these differences occur at certain DNA sites (SNP loci). Autosomal SNPs are biparentally inherited, meaning that one allele is inherited from the mother and the other allele from the father, while mtDNA SNPs are maternally inherited and Y-chromosomal SNPs are paternally inherited (Waits 1999). This marker is co-dominant with a biallelic nature and it is less prone to some amplification errors commonly associated with other marker-types, for example null alleles and allelic drop out (Seddon *et al*. 2005). One SNP on its own does not hold much information or statistical power, but a panel that consist of many carefully chosen SNPs often performs well in population genetics studies (Seddon *et al*. 2005; Hauser *et al*. 2011). Additionally, due to the short sequences containing the single nucleotide of interest, SNP-based sequences have a better chance to amplify than for other markers, which is especially important for degraded and low-quality DNA. This makes SNPs an ideal marker for non-invasive studies (Morin *et al*. 2004).

Non-invasive SNP-based studies on wild species (which have not had their genome fully sequenced) have been scarce, partly due to the absence of well-developed SNP panels that are informative for the study species and population in question. In addition, to my knowledge no previous SNP-based population genetic study has been carried out for brown bears. Recently, however, Norman *et al*. (2013) *de novo* developed a panel with 96 high quality SNPs for relatedness studies of the Scandinavian brown bear population. This SNP panel has potential to be useful for a wider range of purposes, such as studies concerning individual identification, natal dispersal, reproductive success, and population- and conservation genetics.

Aim of this study

The last time genetic studies were carried out for brown bears within the southern Swedish population was ten years ago by Tallmon *et al*. (2004), who stated that "*future monitoring of immigration and inbreeding effects are warranted because demographic impacts of inbreeding can be expressed at any time*". My goal with this master thesis is therefore to genetically characterize the southern Swedish brown bear population within the counties of Dalarna and Gävleborg. I will use the newly developed SNP chip by Norman *et al*. (2013) to genotype 434 brown bears on 96 SNP loci, and all analyses will be based on these data. The

aim is to answer four main questions, including: (i) is the level of genetic diversity sufficient and does this population show any signs of inbreeding?; (ii) are any migrants from the northern population present among the samples?; (iii) is the southern population genetically structured?; and (iv) what is the effective population size (N_e), the N_e/N_C ratio, and what number of bears are required for fulfillment of both the short-term MVP criterion of $N_e \geq 50$ or 100 and the long-term MVP criterion of $N_e \geq 500$ or 1 000? My hypotheses are that:

(i) the genetic diversity is high, as it was in the study by Tallmon *et al*. (2004) and Waits *et al*. (2000), despite the bottleneck of about 50 individuals in the 1930s

(ii) there are males from the northern population present among the samples

(iii) there is a lack of genetic structures within the southern population

(iv) the N_e is insufficient due to the low census population size of approximately 800 bears.

Materials and Methods

The study area includes Dalarna and Gävleborg County and covers about 46 300 km^2, of which Dalarna county is 28 200 km^2 and Gävleborg County is 18 100 km^2 (Fig. 2). The number of brown bears in this area are approximately 793 (95% CI: 621 – 1179) (Kindberg and Swenson 2013), which is an estimate based on visual observations by hunters and DNA analyses of approximately 1 785 fecal samples that were collected non-invasively and opportunistically by hunters, staff from the County administrative board, forest officers and volunteers during autumn 2012. All sampled feces came from both adults and juveniles, since cubs are born in the den and emerge with the mother during April/May. These fecal samples were sent to the laboratory of Bioforsk Jord og Miljø (Svanhovd, Norway) for DNA extraction and genotyping, and results showed that the collected feces contained DNA from 434 unique individuals.

Molecular analysis

DNA extracts from these 434 individuals were sent to the laboratory at the Department of Wildlife, Fish and Environmental Studies at the Swedish University of Agricultural Sciences (Umeå, Sweden) for SNP genotyping. Plates for the BioMark (Fluidigm Corporation, San Francisco, USA) were prepared according to the manufacturer's instructions with the exception of number of pre-amplification cycles: 40 cycles were used instead of 14 in the polymerase chain reaction (PCR). This modification was done to improve amplification success of low quantity/quality DNA. To check for consistency in genotype assignment and to detect potential genotyping errors, a set of randomly selected samples were genotyped two or three times (91 and 10 individuals, respectively). Additionally, six blind samples and 19 water controls were included in the SNP genotyping process. Some SNPs from the original panel (Norman *et al*. 2013) proved to be linked and were therefore replaced with unlinked SNPs, so the final panel in this study consisted of 85 autosomal SNPs, four Y-chromosome sex determination markers, three X-chromosome markers and four mtDNA SNPs.

Data analysis

The outcome of the SNP genotyping process was evaluated through calculations of mean call rate for markers on autosomes, X-chromosomes and mtDNA. The call rate indicates how successful the genotype assignment has been. A call rate of 100 % means that all individuals

have been assigned with a genotype at each SNP. In addition, error rate was calculated for samples that had been genotyped two or three times and minor allele frequencies (MAFs) were calculated for each autosomal SNP. The four Y-chromosome markers were used for sex determination.

Genetic diversity and inbreeding

Nuclear genetic diversity was estimated as observed heterozygosity (H_o) and the heterozygosity expected under HWE (H_e) using R vers. 3.1.1 (R Development Core Team 2013). Deviations from Hardy-Weinberg proportions were calculated through an exact test in the software Genepop vers. 4.3 (Rousset 2008) using a Markov Chain method with default settings of 10 000 dememorization steps, 20 batches and 5 000 iterations per batch. The within population inbreeding coefficient F_{IS} was calculated through one locus estimates following standard ANOVA as in Weir and Cockerham (1984) using Genepop vers. 4.3 (Rousset 2008). F_{IS} values ranges from -1 to 1 and negative values indicate an excess of heterozygotes, while a positive value indicates an excess of homozygotes.

Migrants from the northern Swedish population

First generation migrants were identified by manually screening all four mtDNA markers. Individuals that differed from the western mtDNA haplotype at all four SNPs were considered as belonging to the eastern mtDNA haplotype and the northern population. In addition, the reproductive success of migrants was evaluated through autosomal SNPs (n = 85). SNPs with mean minor allele frequencies that differed by more than two standard deviations from the median between the validation panel (Norman *et al*. 2013) and samples from the southern population were considered as potentially informative. These SNPs were manually screened and individuals with minor alleles were considered possible migrants.

Population structure

All individuals (n = 434) and autosomal SNPs (n = 85) were incorporated in the following two analyses. First, a Bayesian clustering analysis with a Markov Chain Monte Carlo (MCMC) approach was applied in order to test for the number of genetic clusters (*K*) within the southern population. The software STRUCTURE vers. 2.3.4 (Pritchard *et al*. 2000) was used with a parameter set of 100 000 burnins and 500 000 MCMC repetitions with 20 iterations each for values of *K* ranging from 1 to 5, and the admixture model. The number of *K*s was evaluated according to two methods: (i) the lowest lnP(D) as in Pritchard *et al*. (2000), and (ii) the ad hoc statistic delta *K* (ΔK) method as in Evanno *et al*. (2005). Second, an additional approach to check for possible clustering patterns within the southern population was done through a principal component analysis (PCA) by using R vers. 3.1.1 (R development Core Team 2013).

Effective population size, N_e/N_C ratio and MVPs

N_e was estimated using the bias-corrected version of the method based on linkage disequilibrium (LD) (Hill 1981; Waples 2006; Waples and Do 2010), as implemented in NeEstimator V2.01 (Do *et al*. 2014). All bears (n = 434) and loci (n = 85) were included in the calculation. The samples were mixed-aged with both juveniles and adults. The precision of the estimate is presented as a confidence interval with a 95% confidence limit. Calculations of N_e/N_C ratios are based on the known number of individuals (n = 793) in Dalarna and Gävleborg during 2012 (Kindberg and Swenson 2014). The population sizes that are required in order to fulfill either the short-term ($N_e \geq 50$ or 100) or the long-term MVP ($N_e \geq 500$ or 1 000) were calculated by dividing 50 and 100 as well as 500 and 1 000 with the N_e/N_C ratio.

Results

Data analysis

434 brown bears were successfully SNP genotyped, yielding 244 females and 190 males (Table 1). Two individuals had exactly the same genotypes, so the actual number might be only 433 individuals, however, this 'extra' individual will likely not affect the results in this thesis and therefore it was not removed. The mean call rate was 0.997%. A total of 101 samples were genotyped two or three times and only three genotyping errors were found out of 19 504 assigned genotypes, which corresponds to an error rate of 0.00015%. The mean MAF for autosomal SNPs (n = 85) was 0.373. See the Appendix for specific values of each SNP.

Table 1. The columns 'Females', 'Males' and 'Sum' specify the number of SNP genotyped bears from each County. The bottom row specifies the total number of SNP genotyped bears.

County	Females	Males	Sum
Dalarna	126	90	216
Gävle	118	100	218
Total	244	190	434

Genetic diversity and inbreeding

The mean H_o (0.45) was lower than H_e (0.49), however, the deviation from HWE was not significant (p = 0.0716) except for five loci (SNP 120, 128, 150, 183 and 223). The mean F_{IS}-value was negative (-0.0014) and indicates a slight excess of heterozygotes. See the Appendix for specific values of each SNP.

Migrants from the northern Swedish population

A total of seven males had different genotypes at all four mtDNA loci and thus a different mtDNA haplotype. This means that these individuals do not belong to the western mtDNA lineage. Instead, they most likely belong to the eastern mtDNA lineage and are first generation migrants from the northern population. For sampling locations of these males, see Fig. 2. Three autosomal SNPs (SNP 168, 181 and 221) had mean minor allele frequencies that differed by more than two standard deviations from the median, and a total of sixteen individuals (males = 9, females = 7) had minor alleles at two out of three of these loci. However, it is not possible to state that these sixteen individuals actually are offspring from migrants due to the fact that only three SNPs have low statistical power.

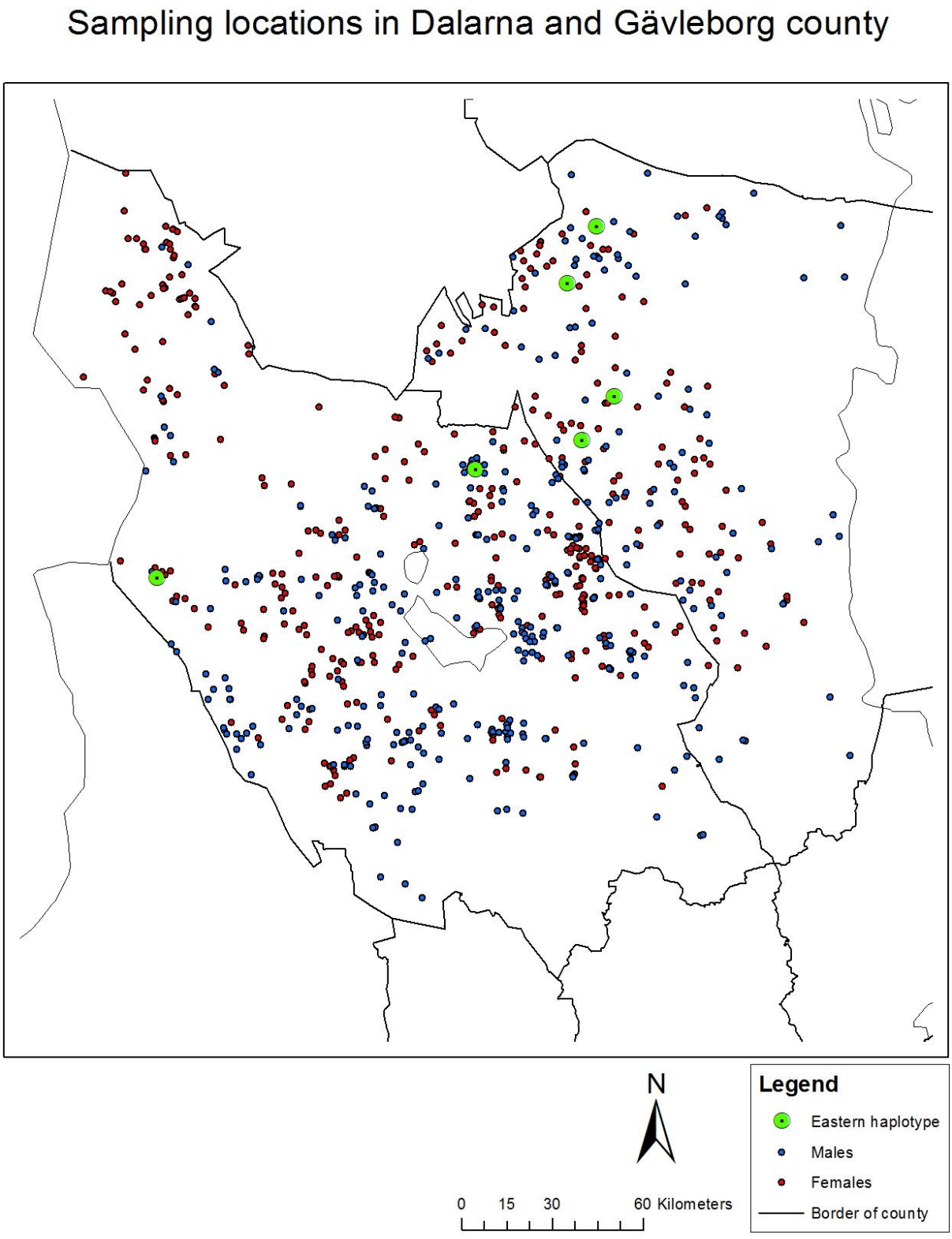

Figure 2. This map shows the sampling locations in Dalarna and Gävleborg County. Several individuals were sampled multiple times and approximately 60 individuals did not have any coordinates associated with any of the sample sites. Additionally, only six out of seven males from the northern population with the eastern mtDNA haplotype are shown. This is because one male did not have any coordinates associated with the sample.

Population structure

The analysis of genetic substructures performed in STRUCTURE was evaluated through STRUCTURE Harvester (Earl and vonHoldt 2012) and resulted in two possible scenarios dependent on the evaluation method: the method based on the lowest lnP(D) (Pritchard *et al*. 2000) suggests one population (K = 1) as the most likely scenario, while the ad hoc statistic delta K (ΔK) method by Evanno *et al*. (2005) suggests that there are three subpopulations (K = 3) present among the samples (Fig. 3). The PCA resulted in one loose cluster (Fig. 4).

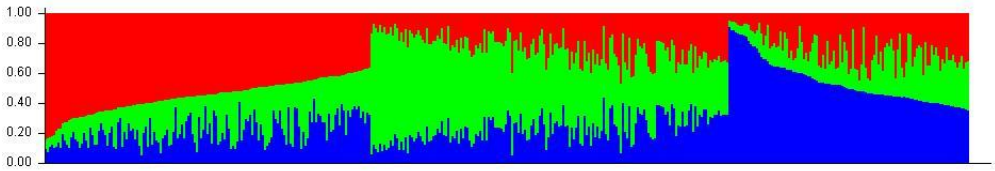

Figure 3. A barplot from STRUCTURE output, sorted by Q. The Evanno-method suggested K = 3 as the most likely scenario and this plot shows how individuals has been assigned to each of these potential clusters.

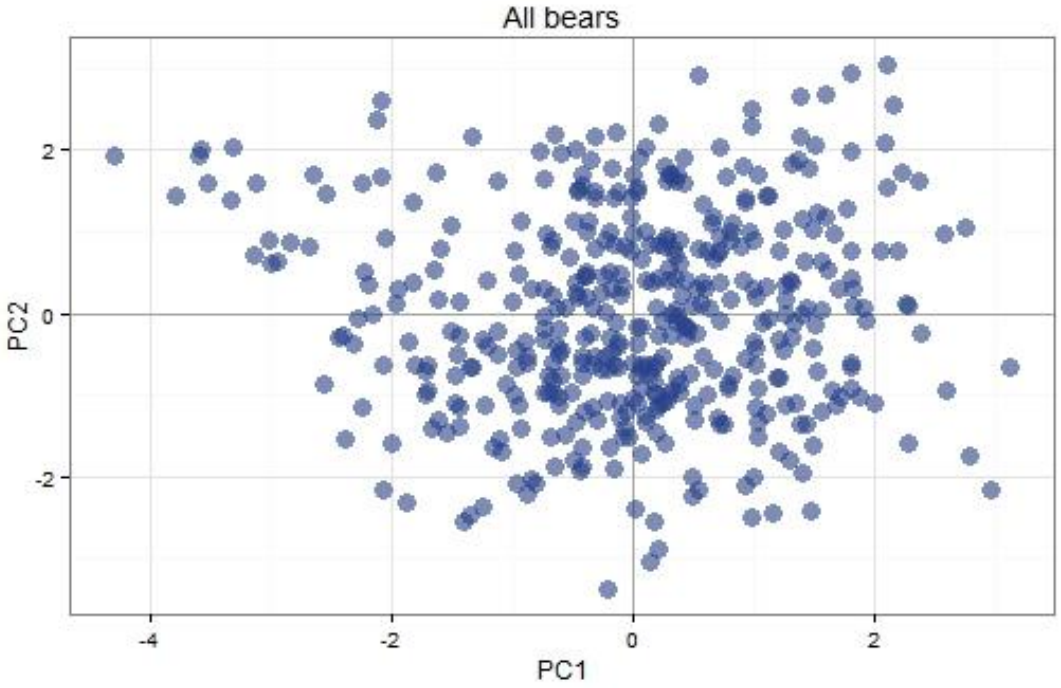

Figure 4. This PCA plot shows the clustering patterns of autosomal SNPs (n = 85) and all bears (n = 434). Each spot is equivalent to a unique bear and overlapping spots with a darker color indicates genetic similarities.

Effective population size, N_e/N_C ratio and MVPs

Calculations of the effective population size based on the LD method resulted in a N_e of 74.4 individuals (95 % CI: 69.1 to 81.1). The estimate of the ratio of effective-to-census population size (N_e/N_C) resulted in 0.094 (0.06 to 0.13). Table 2 shows the number of bears that are required in order to fulfill the different MVP criterions.

Table 2. The total number of bears that are required for fulfillment of the different MVP criterions. The precision of the estimate is presented as a confidence interval with a 95% confidence limit (values within parentheses).

	Short-term MVP		Long-term MVP	
	$N_e \geq 50$	$N_e \geq 100$	$N_e \geq 500$	$N_e \geq 1\,000$
# bears (95% CI)	532 (385 to 833)	1 064 (769 to 1 667)	5 319 (3 846 to 8 333)	10 638 (7 692 to 16 667)

Discussion

In this thesis, I considered four main questions related to the southern Swedish brown bear population; genetic diversity and inbreeding, the number of migrants, population structure and effective population size. As for the different hypotheses I found that:

(i) the southern population did not deviate from HWE and that the observed heterozygosity is close to the expected heterozygosity, thus indicating sufficient levels of genetic diversity. This is in line with previous studies by Waits *et al*. (2000) (H_o: 0.76 and H_e: 0.66) and by Tallmon *et al*. (2004) (see Table 1 in their paper) who both found high levels of genetic diversity. These results suggest the population has recovered since the bottleneck during the 1930s where an estimated 50 individuals survived. Additionally, the slight excess of heterozygotic loci further suggests that this population does not suffer from inbreeding

(ii) a total of seven male immigrants from the northern population were identified. The male-mediated gene flow might reduce or prevent possible inbreeding effects since the mean F_{IS} indicated a slight excess of heterozygotes

(iii) based on results from STRUCTURE and the PCA, no apparent genetic structures were found, which could be due to the high mobility among males

(iv) the N_e resulted in 74.4 individuals which is not enough for long-term viability according to the different MVP-criteria. This number should ideally increase to between 100 and 1 000, which could be enabled through an increase of the number of individuals and further gene flow.

Migrants from the northern Swedish population

Few studies have examined the direct number of migrants between the northern and the southern population of brown bears in Sweden. For example, both Taberlet *et al*. (1995) and Norman *et al*. (2013) found two males and Waits *et al*. (2000) found four males within the southern population who had the eastern mtDNA haplotype and thus belonged to the northern population. This study provides further support to the theory about male-mediated gene flow through the findings of seven first generation male migrants within the southern population who had the eastern mtDNA haplotype and thus origins from the northern population. Although the reproductive success is unknown, it is presumably right to suggest that gene-flow between the northern and the southern population does occur. In this case, the male-mediated gene flow from the northern population will cause a spread of genes and alter the allele frequencies within the southern population. This is important since gene flow is a process that increases both the genetic diversity and the effective population size.

It is worth mentioning that there appears to be a total absence of female migrants. This is likely due to the fact that females are prone to stay in the vicinity of their birth area (Taberlet *et al*. 1994). However, a study by Støen (2006) showed that approximately four out of ten females actually do migrate, most likely to avoid intra-sexual competition for breeding sites, males or other resources. Yet, so far no females have been observed or identified on the 'wrong' side of the contact zone. Since mtDNA is maternally inherited and females seem to avoid crossing the contact zone for some reason, introgression of mtDNA is unlikely to occur, which is also proposed in a paper by Taberlet *et al*. (1994). This means that the western and the eastern mtDNA lineages in Sweden will be kept distinct from each other.

Further, there are some potential scenarios that can make introgression of mtDNA a more likely event in the future, for example, if the population size and abundance of bears within a core area increases or suitable habitats are fragmented/ destroyed thus forcing the bears to relocate. These scenarios might result in an increased movement among bears as they seek suitable home ranges, potential breeding partners, avoidance of inbreeding or avoidance of intra-sexual competition for resources. With time, this could lead to expanding core areas and a blurred contact zone with females on the 'wrong' side. Expanding core areas is in fact not an unlikely scenario and seems to be an ongoing process. For example, the excessive hunting and loss of suitable habitats during the 19^{th} and 20^{th} century led to a population size of 130 bears and the formation of four small female core areas, which later were revised to include only three (but larger) core areas when the population size had grown to about 3 300 bears (Fig. 1a) (Waits *et al*. 2000; Norman *et al*. 2013).

Population structure

My results based on STRUCTURE suggest either one or three subpopulations; however, the PCA results in only one cluster making it the more likely scenario. These results suggest that the southern population does not have any distinct subpopulations and that mating is random, although, we cannot rule out the possibility that there is some fine-scale genetic structuring going on.

Effective population size

Tallmon *et al*. (2004) calculated the effective population size of the southern Swedish brown bear population (based on samples from Dalarna, Gävleborg and lower parts of Jämtland County) through the temporal method and estimated N_e to 44.8 (95% CI: 30.9 to 73.2) at a time when the population consisted of about 700 bears. The calculations in this thesis are based on the LD method and resulted in a N_e of 74.4 (95% CI: 69.1 to 81.1) with a census population size of 793 bears (95% CI: 621 – 1 179) (Kindberg and Swenson 2014). It is important to keep in mind that this new N_e estimation is valid for the year 2012 and one or a few generations before that since the LD method primarily provide information about N_e in the parental generation (Waples 2005). The southern Swedish brown bear population has thus not only increased in numbers during the last ten years, but it has also become more viable in the long-term since the time of the study by Tallmon *et al.* (2004). However, these two approaches for estimating N_e are quite different so it would be interesting to estimate N_e through the temporal method as well. Further, the average N_e/N_C ratio for brown bears is 0.037-0.19 as reported by Paetkau *et al*. (1998) and 0.06-0.14 as reported by Tallmon *et al*. (2004). My results are in agreement with their findings with a N_e/N_C ratio of 0.094 and a range between 0.06-0.13.

To manage the southern population according to the short-term criterions of N_e 50 or 100, the number of bears required would be approximately 533 or 1 066, respectively. The southern

population falls almost exactly in between these two short-term MVP values with a census size of 793 bears and a N_e of 74.4. With respect to this, inbreeding could be a potential problem in the future, unless gene flow from the northern population increases the N_e. For fulfillment of the long-term MVP criterions of $N_e \geq 500$ or 1 000, however, approximately 5 319 or 10 638 individuals are necessary. Moreover, the population size that is required to fulfill the long-term MVP number of $N_e \geq 500$ is lower (5 319 bears) than the one estimated by Nilsson (2013), who based his calculations on results from Tallmon *et al*. (2004) and suggested that a total of 6 838 bears within Dalarna and Gävleborg County is required. As for now, the southern population has high levels of genetic diversity, no signs of inbreeding and the seven male immigrants indicate gene flow from the northern population into the southern population. Due to all of these signs of a genetically healthy population, one question inevitably arises; are the recommendations of N_e and the calculations of MVP-sizes even applicable to K-strategists and top predators such as the brown bear? This needs to be examined further.

SNPs as a marker when using low-quality DNA

The SNP genotyping process resulted in only three genotyping errors and thus an extremely low error rate of 0.00015%. Other markers are generally associated with considerably higher error rates. For example, Bonin *et al*. (2004) reviewed the error rates for microsatellites and amplified fragment length polymorphisms (AFLPs), two co-dominant molecular markers, and found that they ranged between 0.8-2.0% and 2.0-2.6%, respectively. Therefore, the low error rates in this study provide further support for the use of SNPs in non-invasive studies where low-quality DNA is more of a rule than an exception.

Implications for management

Management of large carnivores such as brown bear is not a straight-forward task. Different aspects must be taken into consideration, including for example, keeping the population at low densities in areas where farmers keep their domestic animals and where Sami have their reindeer so as to avoid conflicts, or relocating an individual if found in the vicinity of cities or other settlements due to the risk of encounters between humans and animals. The brown bear population is not threatened and thus not on Sweden's red list. However, Sweden has signed the EU habitats directive, which is key in Europe's nature conservation policy and involves preservation of endangered plants and animals, including all large carnivores. This means that bear hunting is strictly regulated through a yearly licensed hunt during the dates between 21st August and 15th October, or until the County specific quota has been filled, although, protective hunting of problematic individuals can be permitted year-round by the County administrative board. Apart from all different aspects that involve socio-politics, decision makers should also take genetic diversity into consideration as it is one of three levels of biodiversity recognized by the IUCN. It is necessary to think about how hunting affects the genetic composition of the species, and how fragmentation of suitable habitats alters the genetic structures within a population when gene flow among populations might be compromised.

The suggested increase in population size from the present number of approximately 800 to over 3 800 might be problematic for two main reasons; (i) It would require extensive areas of suitable habitats, and: (ii) it would likely increase the number of bear encounters with humans and animals. Today there is about 0.017 bears/km^2 in Dalarna and Gävleborg County and if the number of bears were allowed to increase to 3 800, there would be about 0.082 bears/km^2.

Conclusions

Based on the results in this thesis, the southern Swedish population of brown bears has high levels of genetic diversity and there are no indications of inbreeding depression. Further, this population has no apparent genetic structures, which suggests that mating is random. Future studies should try to detect patterns of fine-scale structuring since previous research has identified a preference among females for large and old males (Steyaert *et al*. 2012), which suggests that mating is not random at all. The findings of seven immigrant males are in agreement with the theory about male-mediated gene flow between the northern and the southern population, which is positive since gene flow maintains high genetic diversity and increases the N_e. It is not known, however, exactly how successful immigrant males from the northern population are in reproducing, and it would be of great interest to know more about these males, such as age, body condition and what chance they stand in competition toward other males. The genetically effective population size is 74.4, which is a number that has increased since the N_e of 44.8 in 2004. This might be due to increased male-mediated gene flow between the northern and the southern population. Since the southern population seems to be healthy from a genetic perspective, future studies could focus on to examine whether the different recommendations of N_e and MVP criterions are at all applicable to top predators with long generation times.

This is the first time ever that a panel of single nucleotide polymorphism markers has been used to genetically characterize brown bears through non-invasively sampled DNA. The future use of SNPs as molecular marker in non-invasive studies is bright and promising due to the high genomic coverage, low genotyping error rate and good reproducibility among laboratories.

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Appendix

Values of observed (H_o) and expected (H_e) heterozygosity for each SNP and associated p-values with a significance level set at 0.05. Rows with bold text indicate loci in Hardy-Weinberg disequilibrium. Negative values in the F_{IS}-column indicate an excess of heterozygotes, while a positive value indicates an excess of homozygotes. The column MAF shows the minor allele frequencies for each SNP.

SNP	H_o	H_e	p-value	F_{IS}	MAF
s101	0.49	0.50	0.923	0.0092	0.481
s102	0.45	0.46	0.680	0.0232	0.362
s104	0.45	0.48	0.200	0.0656	0.410
s105	0.52	0.50	0.344	-0.0504	0.457
s111	0.44	0.42	0.418	-0.0421	0.300
s112	0.50	0.48	0.619	-0.0289	0.400
s114	0.49	0.48	0.693	-0.0228	0.398
s115	0.48	0.48	0.923	0.0057	0.410
s116	0.38	0.40	0.279	0.0533	0.278
s118	0.52	0.49	0.328	-0.0486	0.427
s119	0.45	0.50	0.058	0.0941	0.478
s120	**0.47**	**0.42**	**0.018***	-0.1116	0.306
s125	0.54	0.50	0.074	-0.086	0.483
s127	0.50	0.50	0.923	-0.008	0.477
s128	**0.45**	**0.50**	**0.050***	0.0966	0.460
s129	0.49	0.48	0.843	-0.0143	0.402
s131	0.41	0.40	0.729	-0.0208	0.275
s133	0.54	0.50	0.129	-0.0783	0.487
s134	0.47	0.49	0.315	0.0479	0.416
s136	0.48	0.50	0.336	0.0494	0.475
s141	0.53	0.50	0.292	-0.0539	0.468
s145	0.42	0.40	0.277	-0.058	0.272
s147	0.50	0.48	0.682	-0.0231	0.406
s150	**0.43**	**0.50**	**0.003****	0.1452	0.452
s156	0.52	0.50	0.380	-0.0417	0.482
s159	0.47	0.44	0.188	-0.0657	0.320
s160	0.49	0.50	0.699	0.0205	0.454
s162	0.45	0.46	0.463	0.0369	0.365
s164	0.50	0.50	0.850	-0.0105	0.499
s165	0.40	0.43	0.264	0.057	0.307
s166	0.52	0.49	0.278	-0.0565	0.419
s168	0.38	0.40	0.237	0.0615	0.279
s169	0.54	0.50	0.079	-0.0841	0.479
s170	0.33	0.31	0.169	-0.0695	0.191
s172	0.52	0.50	0.560	-0.0297	0.486
s175	0.28	0.29	0.514	0.0306	0.174
s176	0.48	0.50	0.523	0.0333	0.457
s177	0.40	0.37	0.126	-0.0793	0.242
s179	0.51	0.47	0.147	-0.0687	0.388
s180	0.54	0.50	0.127	-0.0779	0.498
s181	0.47	0.50	0.156	0.0738	0.483
s183	**0.53**	**0.47**	**0.006****	-0.1268	0.375
s184	0.46	0.45	0.915	-0.0099	0.347
s186	0.50	0.50	0.927	-0.0058	0.497

s189	0.50	0.49	0.919	-0.0056	0.429
s191	0.48	0.49	0.623	0.024	0.431
s193	0.42	0.45	0.214	0.0645	0.342
s195	0.26	0.26	1.000	-0.0049	0.154
s199	0.47	0.47	0.920	-0.0075	0.378
s200	0.50	0.47	0.310	-0.0503	0.382
s201	0.23	0.25	0.083	0.0848	0.147
s202	0.44	0.48	0.056	0.0933	0.409
s203	0.48	0.49	0.840	0.01	0.416
s204	0.50	0.48	0.470	-0.0368	0.396
s205	0.41	0.42	0.561	0.0319	0.302
s206	0.52	0.49	0.198	-0.065	0.424
s207	0.38	0.35	0.230	-0.0644	0.230
s209	0.47	0.50	0.331	0.0507	0.459
s211	0.42	0.45	0.120	0.0753	0.339
s212	0.48	0.46	0.522	-0.035	0.363
s213	0.45	0.47	0.414	0.0396	0.372
s214	0.42	0.42	0.911	0.0099	0.302
s217	0.44	0.45	0.829	0.0136	0.341
s218	0.42	0.42	0.906	0.0095	0.301
s219	0.49	0.49	0.923	0.006	0.417
s220	0.44	0.43	0.911	-0.0088	0.315
s221	0.23	0.23	0.835	0.0085	0.133
s222	0.49	0.50	0.842	0.0097	0.456
s223	**0.45**	**0.50**	**0.042***	0.0979	0.464
s225	0.51	0.49	0.432	-0.0398	0.419
s226	0.40	0.42	0.139	0.0713	0.306
s227	0.39	0.40	0.251	-0.0621	0.275
s228	0.51	0.46	0.083	-0.0843	0.368
s230	0.42	0.44	0.267	0.0556	0.328
s231	0.47	0.46	0.833	-0.0117	0.364
s234	0.42	0.45	0.244	0.0572	0.338
s237	0.50	0.50	1.000	-0.0005	0.454
s239	0.47	0.46	0.520	-0.032	0.354
s240	0.44	0.43	0.642	-0.0245	0.309
s241	0.34	0.33	0.466	-0.0389	0.209
s244	0.52	0.50	0.460	-0.0373	0.480
s245	0.48	0.48	0.768	0.0185	0.408
s250	0.30	0.33	0.105	0.0851	0.204
s251	0.48	0.48	0.922	0.0073	0.400
s253	0.37	0.39	0.324	0.0494	0.263

*= p-value < 0.05, **= p-value < 0.01

SENASTE UTGIVNA NUMMER

2013:17 The cost of having wild boar: Damage to agriculture in South-Southeast Sweden.
Författare: Tomas Schön

2013:18 Mammal densities in the Kalahari, Botswana – impact of seasons and land use.
Författare: Josefine Muñoz

2014:1 The apparent population crash in heath-hares *Lepus timidus sylvaticus* of southern Sweden – Do complex ecological processes leave detectable fingerprints in long-term hunting bag records?
Författare: Alexander Winiger

2014:2 Burnt forest clear-cuts, a breeding habitat for ortolan bunting *Emberiza hortulana* in northern Sweden?
Författare: Cloé Lucas

2014:3 Movement ecology of the golden eagle *Aquila chrysaetos* and the semi-domesticated reindeer *Rangifer tarandus*.
Författare: Mattias Nilsson

2014:4 Tick burden in neonatal roe deer (*Capreolus capreolus*): the role of age, weight, hind foot length, and vegetation and habitat on bed sites
Författare: Evelina Svensson

2014:5 Effects of tree retention on cavity-nesting birds in northern Sweden.
Författare: Eva Domingo Gómez

2014:6 Utvärdering av lockmedel för mark-levande predatorer under midvinter-månader i Norrbottens inland.
Författare: Martin Johansson

2014:7 Role of cervids and wild boar on the presence of tick-borne encephalitis virus in Sweden.
Författare: Carmelo Gómez Martínez

2014:8 Full Circle: Upstream and downstream migration of Atlantic salmon (*Salmo salar*) in the northern Swedish river Vindelälven.
Författare: Raven Grandy-Rashap

2014:9 Nyckeltal för älg och fodertillgång på tall Pinus sylvestris och rönn Sorbus aucuparia.
Författare: Mikael Åkerblom Andersson

2014:10 Rissepareringens effekter på viltets nyttjandegrad av GROT.
Författare: David Rehmberg

2014:11 Fysiska strukturer i Umeälvens gamla älvfåra och dess inverkan på laxsmoltens utvandringsframgång.
Författare: Viktoria Tegenfeldt