Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

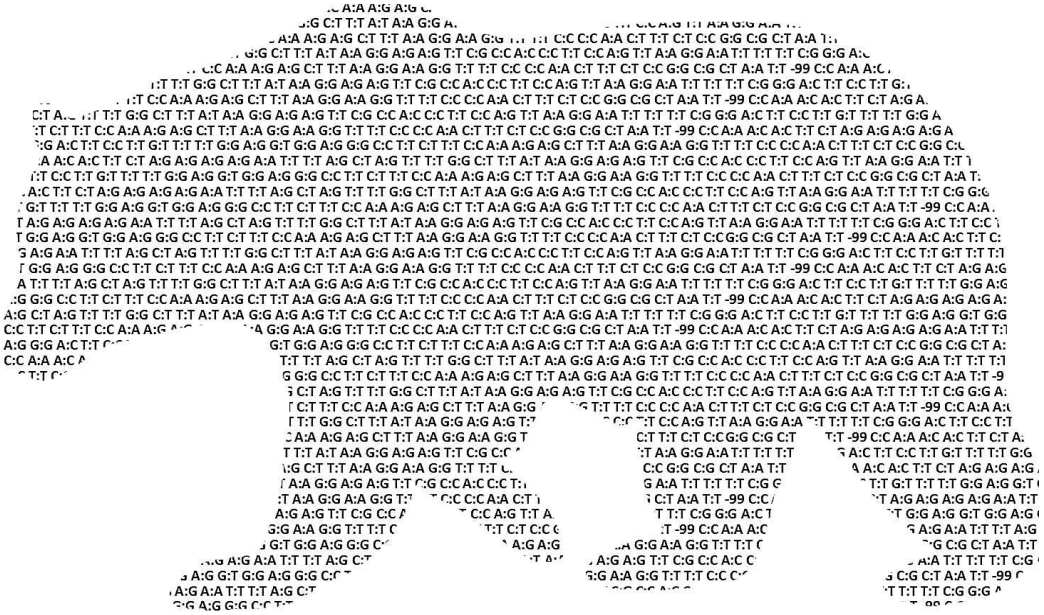
Faculty of Forest Science

BEYOND GENOTYPE

Using SNPs for pedigree reconstruction-based population estimates and genetic characterization of two Swedish brown bear (Ursus arctos) populations

Struktur och storlek hos två björnpopulationer (Ursos arctos) från SNP-baserade släktträdsanalyser

Robert Spitzer



Examensarbete i ämnet biologi
Department of Wildlife, Fish, and Environmental studies
Umeå
2015

BEYOND GENOTYPE

Using SNPs for pedigree reconstruction-based population estimates and genetic characterization of two Swedish brown bear (Ursus arctos) populations

Struktur och storlek hos två björnpopulationer (Ursos arctos) från SNP-baserade släktträdsanalyser

Robert Spitzer

Supervisor: Göran Spong, Dept. of Wildlife, Fish, and Environmental Studies

Assistant supervisors: Anita Norman, Dept. of Wildlife, Fish, and Environmental Studies

Examiner: Jean-Michel Roberge, Dept. of Wildlife, Fish, and Environmental Studies

Credits: 30 HEC
Level: A2E
Course title: Master degree thesis in Biology at the Department of Wildlife, Fish, and Environmental Studies
Course code: EX0764
Programme/education: Management of Fish and Wildlife Populations – Master`s Programme

Place of publication: Umeå
Year of publication: 2015
Cover picture: Robert Spitzer
Title of series: Examensarbete i ämnet biologi
Number of part of series: 2015:9
Online publication: http://stud.epsilon.slu.se

Keywords: molecular ecology, SNPs, pedigree reconstruction, rarefaction, population estimates

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Forest Science
Department of Wildlife, Fish, and Environmental Studies

1 Abstract

Reliable population estimates are an important aspect of sustainable wildlife management but usually difficult to obtain for rare and elusive large carnivores. I tested a new method developed by Creel and Rosenblatt (2013) to estimate the population size of two Swedish brown bear (*Ursus arctos*) populations. The Creel-Rosenblatt estimator (CRE) projects beyond the count of genotypes by including individuals that were inferred from the pedigree as well as undetected individuals into the population estimates. Using a recently developed panel of 96 single nucleotide polymorphisms (SNPs), hunter-collected fecal samples were genotyped for reconstructing pedigrees. Based on 434 genotypes from Dalarna-Gävleborg and 265 from Västerbotten, the CRE population estimates ($\widehat{N}$ = 623 for Dalarna-Gävleborg, $\widehat{N}$ = 404 for Västerbotten) fell within the 95% CI of the official estimations. As predicted in simulations, the CRE performed best if the sampling intensity was >40%. At present no agreed method exists to calculate confidence limits for CRE estimates. As an interim solution I suggest using genotype count and maximum inference from the pedigree for assigning lower and upper bounds. To complement the work by Fahlen (2014) on conservation genetics for the Dalarna-Gävleborg population, I used the genetic and spatial data from Västerbotten in subsequent analyses to assess genetic health, population structure and movement patterns. I found slight indication of inbreeding (F_{is} = 0.025), male-mediated gene flow from one migrant of the western lineage and possible segmentation into two major sub-populations. Non-invasive genetic sampling in combination with pedigree reconstruction and spatial analysis shows promising potential for future studies of population dynamics, dispersal and reproductive success.

2 Introduction

Part one of Charles Krebs (1999) renowned book *Ecological Methodology* opens with perhaps the most foundational question of all wildlife ecology studies: "How many are there?" (p. 17). While researchers and managers alike are generally in agreement about the importance of the question, finding an answer often proves to be tantalizingly difficult. In addition, the implications of getting it wrong are potentially severe because reliable estimates of population size and trends form the basis of key management decisions. Common examples are the setting of harvesting quotas (Wilson and Delahay 2001) or the IUCN Red-listing of endangered species (Vié et al. 2009). Ironically, solid estimates are especially difficult to obtain for rare and elusive species which frequently also happen to be the ones of highest concern. This includes most large carnivores (Creel and Rosenblatt 2013, Kindberg et al. 2009). They generally occur in comparatively low numbers across large home ranges, making them expensive to study (Zuercher et al. 2003) and are often ambiguously or outright controversially viewed by the public (Ericsson and Heberlein 2003, Kellert et al. 1996). As a consequence, many carnivore populations remain poorly understood due to a paucity of data (Karanth and Chellam 2009) or bias arising from public pressure (Balme et al. 2014, Zimmermann et al. 2010).

The Scandinavian brown bear (*Ursus arctos*) is a typical example for the challenges surrounding large carnivore research but also unique in the sense that it is arguably one of the best studied carnivores in Europe. The Scandinavian Brown Bear Research Project (SBBRP), a collaboration between Swedish-Norwegian and international researchers, presently lists 18 doctoral theses and 175 publications in peer-reviewed journals about the species on their website (Björnprojektet 2015).

After the last ice age, brown bear recolonization of Sweden probably occurred from around 11,000 BP until 6,000 BP when the vegetation started to resemble its present state (Hewitt 1999). Studies of maternally-inherited mitochondrial DNA (mtDNA) have shown that brown bears in Sweden belong to two genetically distinct lineages with approximately 7% differentiation between them (Taberlet and Bouvet 1994). The western lineage, found in south-central Sweden, originated from the Iberian refugium (today's France and Spain) whereas the eastern lineage, found throughout northern Sweden can be traced to Karelia in Russia (Taberlet and Bouvet 1994). Norman et al. (2013) reported a sub-division into three mitochondrial-based haplotypes: South (western lineage), North A and North B (eastern lineage). At present the two lineages remain largely separated around a well-documented contact zone at the height of Östersund in central Sweden (Taberlet et al. 1995). Gene flow across the contact zone by migrants has been recorded several times for males but not for females (Fahlen 2014, Taberlet et al. 1995, Waits et al. 2000). Monitoring population genetics is especially important in case of the western mtDNA haplotype (southern population) which is only found in Europe whereas the eastern haplotype is also prevalent in Asia and North America (Hirata et al. 2013, Korsten et al. 2009, Saarma et al. 2007, Waits et al. 2000). Intense hunting pressure during the 19th century led to a rapid decline of the Swedish bear population, resulting in an estimated low of about only 130 individuals in the 1930s (Waits et al. 2000). After the introduction of protective measures, like the removal of bounties, the numbers quickly recovered (Saether et al. 1998, Zedrosser et al. 2011) to an estimated 3,300 individuals today (Kindberg et al. 2011). However, while positive trends are certainly encouraging, they should not be allowed to convey a false sense of security leading to the potentially premature conclusion that a species future is ensured. Corroboration of this point could be seen in the circumstance that at the time of writing the status of the Swedish brown bear population was downgraded from its 2010 rating of 'vital' (livskraftig) to 'near-threatened' (nära hotad) due to a pronounced population decline (Artdatenbanken 2015).

Many methods have been developed to estimate the size and trends of animal populations in the wild, including indices, distance sampling and capture-mark-recapture (CMR) (Sutherland 2006). A crucial aspect of CMR studies is the reliable marking and re-identification of individuals during one or multiple recapture events. However, frequent physical restraint and handling of rare and elusive animals, particularly large predators, is ethically questionable, impractical, costly and potentially dangerous to both animals and researchers. Non-invasively collected DNA samples (e.g. from feces or hair) in combination with rapidly improving molecular techniques offer a promising way to bypass these obstacles (Kohn and Wayne 1997, Swenson et al. 2011, Taberlet et al. 1999, Waits and Paetkau 2005). They also present the unique opportunity to involve the public through citizen science as has been practiced in Sweden with hunter-collected brown bear feces since 2001 (Bellemain et al. 2005). Moreover, genetic methods have the advantage that they allow for analyses beyond genotyping individuals for identification. Common examples include kinship assessment and provenience determination.

Single nucleotide polymorphisms (SNPs; pronounced 'snips') are a fairly novel type of genetic marker (Morin et al. 2004) that is rapidly becoming a powerful tool for studying genetic variation in populations (Brumfield et al. 2003). Compared with microsatellites, another type of frequently used genetic marker, SNPs offer lower error rates from mistyping and allelic dropout (Morin and McCarthy 2007). They are also easily reproducible across laboratories and can have high genomic resolution (Anderson and Garza 2006). Because only short intact sequences (50-70 bp) of DNA are required for successful amplification of SNPs via polymerase chain reaction (PCR), SNPs are especially

suitable when working with degraded DNA as is usually the case with non-invasively obtained samples (Morin et al. 2004). SNPs are commonly found across the genome (Vignal et al. 2002). They occur in DNA wherever a base pair has variable nucleotides at a locus. Usually SNP loci are bi-allelic (Sripichai and Fucharoen 2007), resulting in three (two homozygous and one heterozygous) possible genotypes for that locus. Due to lower statistical power, higher numbers of SNPs than microsatellites have to be used to detect an effect (Morin et al. 2009). In the past this inevitably led to higher costs but recent advancements in sequencing technology have since removed that obstacle (Anita Norman, pers. comm.). As shown in Figure 1 the probability of identity (PID) rapidly declines with as little as 7 loci. The genetic data used in this study was produced with a SNP chip specifically developed for the Scandinavian brown bear which included 85 autosomal SNPs (Norman et al. 2013).

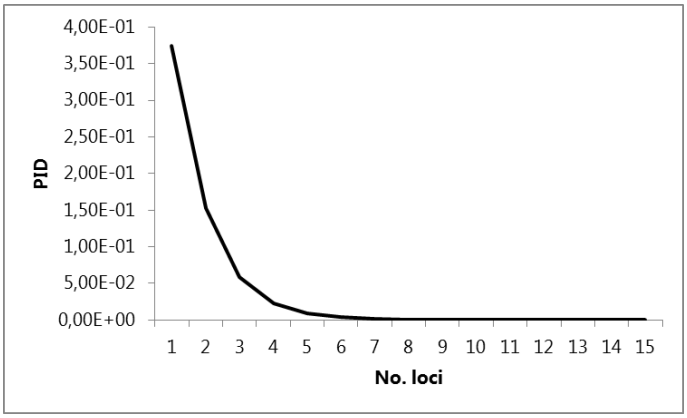


Figure 1 Rapid decline of probability of identity (PID) with increasing number of loci. PID is the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Waits et al. 2001). Calculations were carried out with the software GIMLET version 1.3.3 (Valière 2002) using genomic data of 434 bears from Dalarna-Gävleborg. For better visibility the x-axis was truncated at 15. Across all 85 autosomal loci used in this study, the PID reaches practically zero ($3.13x10^{-34}$).

Using a large number of SNPs effectively eliminates the "shadow effect", where two or more individuals are identified as one due to low numbers or variability of the genetic markers, ultimately leading to an underestimation of the population size (Mills et al. 2001). While genetic markers can help to avoid many pitfalls in population studies, direct genetic census methods are virtually identical to conventional CMR methods in their logic (Creel and Rosenblatt 2013) and prone to some of the same biases. The genotype simply becomes a 'molecular tag' (Schwartz et al. 2007) which replaces traditional means of identification like earmarks or distinct morphological features.

Indirect genetic methods based on pedigree reconstruction include individuals which were not directly sampled and genotyped but rather inferred by their genetic fingerprint into the population estimates. Because an individual must breed and leave offspring in order to become visible in the pedigree, these methods seemed inevitably limited to estimating the number of breeders in a population rather than total population size.

Creel and Rosenblatt (2013) used demographic data of African lions (*Panthera leo*) from Zambia in a simulation model to develop a new, pedigree-based estimator for total population size. Their method, henceforth referred to as the Creel-Rosenblatt estimator (CRE) accounts for individuals that were directly sampled, inferred from the pedigree, as well as "invisible" non-breeders.

Because precise population estimates and large numbers of genetic samples exist for the Swedish brown bear it makes the species uniquely suited for testing the performance of the CRE outside of a simulation environment.
Here, I apply the CRE to estimate the size of two brown bear populations (Dalarna-Gävleborg and Västerbotten). To the best of my knowledge this constitutes the first time that the estimator is applied to empirical population data. Contrary to CMR methods, the CRE requires only one sampling event. This could potentially make the method competitive with regard to cost and effort.
Furthermore, since genetic data is an especially powerful tool when combined with demographic or spatial information (Deyoung and Honeycutt 2005) I also include some analyses based on such data.
In terms of genetically characterizing Swedish bear populations, this thesis builds on the work of Fahlen (2014) who investigated conservation genetics of the Dalarna-Gävleborg bears. Thus, my own work for these parts will primarily focus on the Västerbotten population.
Within the larger framework of the 16th Swedish Environmental Objective "A Rich Diversity of Plant and Animal Life" (Regeringskansliet 2014) this study aims at contributing to the sustainable management of the brown bear by

(I) evaluating the Creel-Rosenblatt estimator as a new census method based on pedigree reconstruction
(II) genetically characterizing the Västerbotten brown bear population and comparing it to Fahlen (2014) results for Dalarna-Gävleborg
(III) using geographic coordinate data recorded by citizen scientists during the collection of fecal samples to investigate spatial population structuring and movements.

Specifically, I hypothesize that

(i) pedigree reconstruction in combination with the Creel-Rosenblatt estimator can be used to estimate total population size from empirical genetic data with similar accuracy as suggested by simulations
(ii) in case of single sampling events the Creel-Rosenblatt estimator performs equal to or better than the alternative methods (direct counts and rarefaction analysis)
(iii) the Västerbotten brown bear population does not suffer from inbreeding
(iv) migrants from the southern population are present in Västerbotten
(v) spatial information from fecal samples can be used to infer population structure and movements
(vi) the pedigree will become increasingly incomplete towards the borders of a sampling area
(vii) reproductive success can be linked to spatial movements.

3 Material and methods

3.1 Study area and data collection

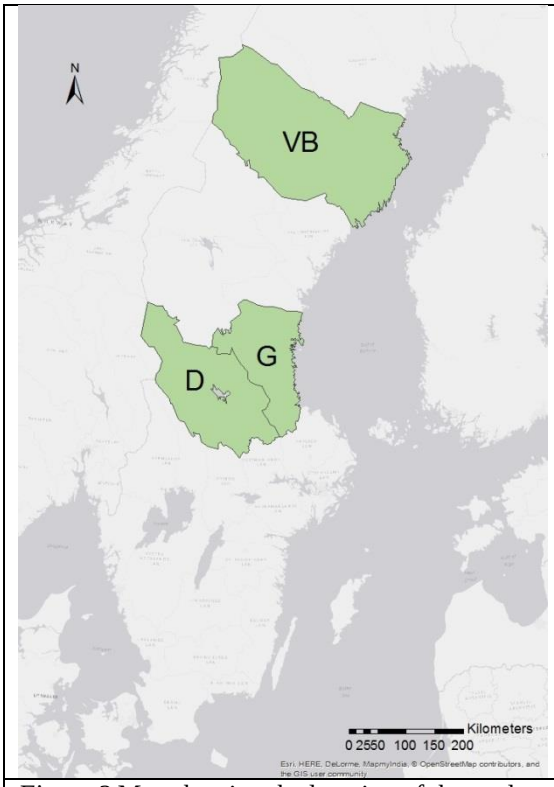


Figure 2 Map showing the location of the study areas; Västerbotten (VB), Dalarna (D) and Gävleborg (G).

The study area (Fig. 2) encompasses the Swedish counties of Dalarna-Gävleborg (ca. 46,300 km²) and Västerbotten (ca. 55,200 km²). The landscape is a mosaic of lakes, wetlands and extensive stands of boreal forest dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). Common deciduous tree species include birches (*Betula* spp), alders (*Alnus* spp) and European aspen (*Populus tremula*).
To the west the area is delimited by the Scandinavian mountain range and in the east by the Baltic Sea. Agriculture decreases with northern latitude and the majority of settlements are situated along the coast (Kindberg 2010). Forestry operations are common throughout the entire area. The southern border of Dalarna-Gävleborg also demarcates the approximate southern limit of the brown bear distribution in Sweden. Dalarna-Gävleborg is home to an estimated number of 793 bears, 95% CI [621, 1179] (Kindberg and Swenson 2013). In 2015 the Västerbotten population was estimated to be 362 bears, 95% CI [310, 459] (Jonas Kindberg, pers. comm.). Fecal samples were collected by volunteers (predominantly moose-hunters) following the protocol of Bellemain et al. (2005) during the periods of August-October 2012 in Dalarna-Gävleborg and August-December 2014 in Västerbotten.
With the samples the hunters enclosed records of the collection date and coordinates of the sampling location and mailed them to the county administration board (in case of the Dalarna-Gävleborg collection) or in Västerbotten directly to the genetics laboratory at SLU in Umeå. Upon arrival samples were stored in 70% Ethanol solution at -20°C as recommended by Frantzen et al. (1998).

3.2 Molecular analysis

DNA extraction from the Dalarna-Gävleborg samples was carried out by Bioforsk, Norway following procedures described by Schregel et al. (2012). In Västerbotten, DNA extraction was performed at SLU using the QIAsymphony SP (Qiagen; Hilden, Germany) robot according to the manufacturer's instructions.
SNPs were genotyped on the Fluidigm Biomark (Fluidigm Corporation; San Francisco, USA) using a 96 SNP panel developed by Norman et al. (2013). Since its first publication the panel has undergone slight modifications (e.g. two linked SNPs were substituted with Y-chromosome SNPs) and now consists of 85 autosomal SNPs, four mtDNA SNPs as well as four Y-chromosome and three X-chromosome markers for sex determination. Each run included negative controls with water in place of DNA. The genotype clusters assigned by the Biomark software were manually screened and loci of questionable cluster affiliation invalidated and removed from subsequent analyses.

Species and sex were assigned according to the following criteria:

bear = mtDNA SNP calls ≥ 3
male = Y-chromosome SNP calls ≥ 3
female = Y-chromosome SNP calls = 0 and X-chromosome SNP calls ≥ 2

Allelic state of the four mitochondrial markers was used to determine the mitochondrial haplotype for each individual, which could then be assigned its provenience (North A, North B or South).
For analyses on the Dalarna-Gävleborg bears I used the same set of consensus genotypes as Fahlen (2014). For Västerbotten I prepared the consensus genotypes by manually comparing multiple genotyping results for the same individual. I started with the genotype that showed the highest PCR success across the 85 autosomal and three X-chromosome loci and substituted "No Call" gaps with the correct allele whenever it could be determined by comparison to the other genotypes of the same individual. Likewise, I counted the mismatches between the allelic state at loci of the putative consensus genotype compared to the other genotypes and estimated the typing error for individuals (TE_{in}) as

$$TE_{in} = \text{No. of mismatches / No. of compared loci.}$$

For example, 8 mismatches in a set of 5 genotyping outputs with 85 loci would result in a typing error for that individual of

$$TE_{in} = 8 / (5*85) = 0.019$$

The overall typing error (TE) used in the pedigree reconstruction corresponds to the mean of the TE_{in} values.

3.3 Data analysis

3.3.1 Pedigree reconstruction

To reconstruct pedigrees I used FRANz software version 2.0.0 (Riester et al. 2009) which uses Markov Chain Monte Carlo (MCMC) simulation for estimating the statistical confidence of parentage inference and produces a number of output files including a maximum likelihood (ML) pedigree. The software requires specifying an approximate maximum number of females and males (Nfmax and Nmmax) to avoid an empty pedigree due to convergence of the Markov Chain to a very high number of individuals (*N*) (Riester et al. 2009). Because I was working under the assumption of an unknown population size I used my estimates from rarefaction analysis (see next chapter) and the sex ratio present in the genotyped samples to set Nfmax / Nmmax to 538 / 419 (Dalarna-Gävleborg) and 249 / 239 (Västerbotten) respectively. I specified the TE as 1.538×10^{-4} for Dalarna-Gävleborg (Anita Norman, pers. comm.) and 0.01 for Västerbotten.
I tested for noise in the software by running three different amounts of randomly selected genotypes from Dalarna-Gävleborg 10 times each in FRANz and compared the parentage and full-sibling (FS) outputs. Likewise, I tested for the effect of an incorrectly specified TE by running the same set of genotypes with the TE settings increasing from the true 1.538×10^{-4} to 0.1.

3.3.2 Population estimates

3.3.2.1 Rarefaction analysis

Rarefaction, often also referred to as accumulation-curve method (ACM), has traditionally been used to estimate species diversity in an area by plotting the cumulative number of newly recorded species against the total number sampled (Colwell and Coddington 1994). The same underlying logic can be applied for estimating population size by substituting the species count with the number of unique individuals/genotypes. One disadvantage of the method is that it often requires a high sampling intensity in order for the accumulation curve to asymptote. Furthermore, Krebs (1999) points out that "the only way one can extrapolate [rarefaction curves] beyond the limits of the samples is by assuming an underlying statistical distribution" (p. 415). A number of equations to fit and extrapolate accumulation data have since been suggested including the following three, which have been used my molecular ecologists (Petit and Valiere 2006):

(1) $y = \frac{ax}{b+x}$ Kohn et al. (1999)

(2) $y = a\left(1 - e^{(bx)}\right)$ Eggert et al. (2003)

(3) $y = a - a\left(1 - \left(\frac{1}{a}\right)\right)^{x}$ Petit and Valiere (2006)

In these models *y* equals the number of unique genotypes, *x* corresponds to the number of samples (genotyped feces), *b* is the rate of decline in the slope and the asymptote *a* represents the estimated population size. I calculated the parameters *a* and *b* through nonlinear iterative regression using the statistical software package JMP Pro version 11.0.0 (SAS Institute).

Model (1) has been used by Kohn et al. (1999) to estimate the size of a coyote (*Canis latrans*) population in California and model (2) by Eggert et al. (2003) to do the same for forest elephants (*Loxodonta cyclotis*) in Ghana.

Model (3) was suggested by Petit and Valiere (2006) who accredit it to a personal communication with D. Chessel. It has since been used by Frantz et al. (2004) for estimating the numbers in a population of Eurasian badgers (*Meles meles*). Simulations by Valière (2002) have shown that Chessel's model tends to underestimate population size whereas the equation suggested by Kohn et al. (1999) is prone to overestimations if the sampling effort is high. The model used by Eggert et al. (2003) appears to fall in between the two. For my analyses I choose the model which best fit my data based on highest R^2 in combination with the lowest values for root average square error (RASE) and average absolute error (AAE).

3.3.2.2 Pedigree reconstruction method (Creel-Rosenblatt estimator)

The pedigree-based estimator suggested by Creel and Rosenblatt (2013) builds on the logic that total population size $(\widehat{N})$ can be estimated as the sum of directly sampled individuals (N_s), breeders whose presence can be inferred from pedigree reconstruction (N_{in}) and the "invisible" non-breeders who are undetectable in the pedigree (N_{iv}).

(1) $\widehat{N} = N_s + N_{in} + N_{iv}$

The steps of the mathematical deduction from this basic assumption to the final estimator (equation 2) are detailed in Creel and Rosenblatt's (2013) paper. The term B_s refers to the sampled breeders (known individuals with descendants in the pedigree).

$$(2) \qquad \widehat{N} = N_s + 2N_{in} - \frac{N_{in}B_s}{N_s + N_{in}}$$

Because the data available for analyses were limited to sex and genotype of individuals, only first-order genetic relationships (parent-offspring dyads and triads or full siblings) could be reconstructed with reasonable certainty.
In my analyses I used the ML pedigree output of the FRANz software which specifies the putative sire and dam of sampled individuals (see Fig. 3).

ID	SIRE	DAM
AC2014-001	AC2014-148	AC2014-223
AC2014-003	*	AC2014-019
AC2014-004	*	*
AC2014-005	AC2014-017	*
AC2014-006	*	*
AC2014-007	*	*
AC2014-008	*	*
AC2014-009	AC2014-161	*

Figure 3 Section of the reconstructed pedigree for bears from Västerbotten. ID refers to a sampled individual; Sire and Dam to its putative parents.

In accordance with the methods used by Creel and Rosenblatt (2013) I inferred individuals as the missing parent in known parent-offspring dyads. In the above example this would correspond to three; one sire and two dams. However, assuming that each missing parent in the dyads constitutes a new individual would most likely be an overestimation because males frequently mate with several females and vice versa (Steyaert et al. 2012). This means, for example, that an inferred sire is likely to be the missing father in several of the mother-offspring dyads. Therefore, I used the improbable scenario in which the number of inferred individuals (N_{in}) equals the number of dyads in the pedigree, only for the estimation of the upper bound of the population estimate.
For a more realistic estimate that accounts for multiple parentages of inferred individuals, I first determined the ratio of all the known individual dams to the known individual sires ($R_{d:s}$). I then used this ratio to infer the missing counterparts from the individual dams and sires in the pedigree dyads (see Box 1 for an example). In this way, the ratio of dams to sires with the inferred individuals included remains the same as it was in the original pedigree. I consider this to be the most likely reflection of the true situation in the population.
Another problem pointed out by Creel and Rosenblatt (2013) is the circumstance that there is no way to ascertain how many of the inferred individuals are actually still alive at the time of the estimate. In their simulations the authors addressed this issue by tracking all individuals and accounting for known deaths. This approach is not applicable to my assumed scenario of a one-time sampling event. Even over the course of several sampling periods it would be difficult to keep track of individual deaths. However, bears are long lived and have low annual mortality rates once they reach their reproductive age at about four to five years (Swenson et al. 2001) as shown in Figure 4. Although the potential bias resulting from an unknown death rate among inferred individuals is probably small, it should not be ignored.

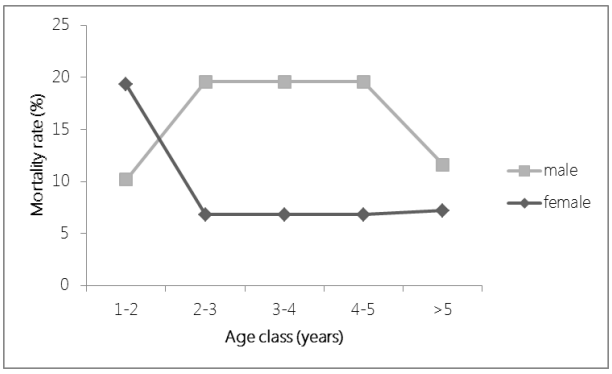


Figure 4 Annual mortality rates of bears in Sweden at different ages based on values taken from Nilsson (2013). While the cause of higher female mortality at age 1-2 is currently still unresolved, the higher male mortality rates from ages 2-5 can be explained by the increased dispersal mobility and less cautious behavior of young males (Bischof et al. 2009). At ages >5 the average annual mortality rate is 7.2% for females and 11.6% for males.

To account for mortality among inferred individuals I assumed them to be of typical breeding age (>5 years) and thus applied average annual mortality rates of 7.2% to inferred dams and 11.6% to sires respectively.

A current limitation of both the Creel-Rosenblatt estimator and rarefaction analysis is the lack of a consensus method for assigning confidence limits. In their rarefaction analyses Bellemain et al. (2005) used 1,000 random iterations of the genotype sampling order, resulting in a range of slightly different asymptotes for the rarefaction curves. In a similar approach I bootstrapped the asymptote *a* of the rarefaction models with 1,000 iterations in JMP Pro (SAS Institute) and calculated 95% confidence limits from the 2.5 and 97.5 percentiles of the bootstrap values (Kindberg et al. 2011). However, this method is more indicative of the sampling variance rather than the true estimator variance (Bellemain et al. 2005).

For the Creel-Rosenblatt estimates I did not assign confidence limits *per se.* Instead, I designated the count of sampled genotypes as the lower bound and set an upper bound by treating N_{in} as equal to the number of dyads in the pedigree. For the estimate of the upper bound I also assumed zero mortality among the inferred individuals.

To test the performance of the CRE at different sampling intensities, I randomly took samples corresponding to 15%, 30% and 45% of the presumed (= estimated by rarefaction) population size. Throughout this thesis, sampling intensity (SI) is denoted as in the following example, taken from the Dalarna-Gävleborg results:

SI_45 (55)

The first number always represents the sample as a percentage of the rarefaction population estimate (45% of 957) while the second number in parentheses corresponds to the percentage in relation to the official population estimate (793 for Dalarna-Gävleborg). Thus, the second value is probably more representative of the true sampling intensity. For Dalarna-Gävleborg *SI_45 (55)* constituted the highest sampling intensity that could be tested because only 434 individuals were sampled (434 / 793 = 0.547). For Västerbotten, the CRE could be tested up to an SI of 73%. In order to compare the CRE to rarefaction analysis, I calculated rarefaction curves for the same sampling intensities.

Box 1 **CRE population estimate for the Dalarna-Gävleborg brown bears**

N_s = 434 [Number of individuals (genotypes) directly sampled]
B_s = 160 [Number of sampled breeders (sires and dams in the pedigree)]

Number of sires in the pedigree: 71
Number of dams in the pedigree: 89

$R_{d:s}$ = 89 / 71 = 1.25 → 1 sire = 1.25 dams

Parent-offspring dyads	**Inferred individuals**	**Mortality correction**
Sires (individuals): 56	56 * 1.25 = 70 (dams)	70 - (70 * 0.072) = 65
Dams (individuals): 65	65 / 1.25 = 52 (sires)	52 - (52 * 0.116) = 46
		N_{in} = 111

$\widehat{N} = N_s + 2N_{in} - \frac{N_{in}B_s}{N_s + N_{in}}$

$\widehat{N} = 434 + 2*111 - \frac{111*160}{434+111}$

$\widehat{N} = 623$

The total number of dyads in the pedigree is 171. Under the assumptions that N_{in} = number of dyads in the pedigree and no mortality, it follows that

Upper bound = $434 + 2*171 - \frac{171*160}{434+171} = 731$.

With the lower bound formed by N_s the CRE population estimate for Dalarna-Gävleborg is

$\widehat{N}$ = 623 [434, 731].

3.3.3 Genetic characteristics

3.3.3.1 Genetic diversity, inbreeding and gene flow

I tested for deviations from Hardy-Weinberg equilibrium (HWE) using the software Genepop version 4.2 (Rousset 2008) with Markov Chain settings of 10,000 dememorizations, 20 batches and 5,000 iterations per batch. Values for expected heterozygosity (H_e) under HWE and observed heterozygosity (H_o) as a measure for nuclear genetic diversity were obtained from the FRANz (Riester et al. 2009) summary output file. I calculated the within population inbreeding coefficient (F_{is}) according to Weir and Cockerham (1984) in Genepop version 4.2 (Rousset 2008).

F_{is} values are bounded by -1 (all individuals heterozygous) and 1 (all homozygous). In other words, a positive F_{is} indicates heterozygote deficiency in comparison with HWE expectations (Waples 2015), which is a sign of inbreeding if that value is significantly different from zero. I tested for statistical significance using a one-sample t-test in JMP Pro (SAS Institute). As for all statistical tests in this study, the significance level was set to $\alpha = .05$.
The test for HWE revealed that in the Västerbotten genotypes 8 of the 85 autosomal loci highly significantly deviated from HWE ($p < .001$). Because this could indicate that they are under selection, they were removed for the final estimation of F_{is}.
Fahlen (2014) reported seven male migrants from the eastern lineage in Dalarna-Gävleborg. I searched for indications of migrant-mediated gene flow from the western lineage (Dalarna-Gävleborg) to Västerbotten by scanning the mtDNA genetic profiles of the Västerbotten samples for the southern haplotype.

3.3.3.2 Population structure

I investigated possible sub-structuring of the Västerbotten population by following similar methods as employed by Fahlen (2014) in her analyses of the Dalarna-Gävleborg bears. First, I used the software package STRUCTURE version 2.3.4 (Pritchard et al. 2000) to test for genetic clusters (*K*) based on the autosomal SNPs (n = 85) of the sampled individuals (n = 265). STRUCTURE uses a Markov Chain Monte Carlo based Bayesian algorithm for the estimation of *K*. Individuals are assigned to each cluster according to their genetic profile at multiple sites (multilocus genotype) in the genome (Pritchard et al. 2010).
Following suggestions by Evanno et al. (2005) I set the length of the burn-in and MCMC to 10,000 and ran 20 iterations for each putative number of *K* (1-10). The results were analyzed in Structure Harvester Web v0.6.94 (Earl and vonHoldt 2012) and the most probable number of genetic clusters determined based on the highest LnP(D) and the delta *K* (ΔK) methods (Evanno et al. 2005).
Second, I used principal component analysis (PCA) in JMP Pro (SAS Institute) to search for clustering patterns.
While the STRUCTURE and PCA analyses solely utilized genetic information, I used GENELAND version 4.0.5 (Guillot et al. 2005) to apply a clustering algorithm that also takes spatial information into account. GENELAND is an extension package for the statistical software R (R Development Core Team 2008) and combines spatial with genetic data in a MCMC process. This approach has the potential to reveal landscape features that interfere with gene flow (fragmentation), which favors the formation of genetically distinct sub-populations. Following recommendations by Guillot et al. (2015) I fixed the maximum rate (rate.max) of the Poisson process at 265 (= individuals in the dataset), the maximum number of nuclei in the Poisson-Voronoi tessellation at 795 (= 3*rate.max) and the uncertainty of the coordinates at 15,000m (= median distance of all known bear movements). I chose an uncorrelated allele frequency model and carried out five independent runs. The run with the highest posterior probability was then post-processed with a burn-in of 200. I inferred the number of clusters *K* from the highest mode of the plot depicting the numbers of population clusters predicted by the MCMC as well as from the maps of posterior probabilities. The spatial clusters suggested by GENELAND were then compared to possible fragmenting landscape features (e.g. roads) using the GIS package ArcMap version 10.2.2 (ESRI 2014).

3.3.4 Spatial investigations

3.3.4.1 The effect of borders on the pedigree

I assumed that the pedigree would become less complete (contain fewer parent-offspring pairs) the further sampling occurred from the core area around the center point of the sampling frame. This is because many breeding individuals might have moved beyond the borders of the sampling area and therefore may have been missed during the sample collection. In both areas, Dalarna-Gävleborg and Västerbotten, the only true population border is the Baltic Sea to the east. To the north and the south, bears occur beyond the borders of the sampling areas. Most interesting is the border to the west, formed by the Scandinavian mountain range. Because mountain terrain can be rugged and difficult to access, the sampling intensity by hunters was lower there than in other areas. If the pedigree were to show similar levels of incompleteness along the western border compared with the "open" borders to the north and the south, it could indicate that bears in the mountains were missed in the sampling. If this were the case it would lead to an underestimation of the population size. If, on the other hand, the mountains form a true border like the Baltic Sea, then the pedigree should be equally complete in both these locations.

From the coordinate data that was provided along with the hunter-collected fecal samples, the median centers of all known locations for an individual were calculated using R (R Development Core Team 2008). Appendix IV contains two maps with these results. The median was considered to be less biased than the mean because of its lower sensitivity to large outliers. Inferring home ranges from the locations of fecal samples is prone to errors but Bellemain et al. (2005) reported that the majority of fecal sites fall inside the home range or within 10 km of it. I determined the center point of the sampling area as the median center of all individual locations using the median center spatial statistics tool in ArcMap. I then sampled the individuals closest to the center point and the four borders (North, South, East & West) respectively at sample sizes of n = 100 for Dalarna-Gävleborg and n = 70 in Västerbotten. The number of samples for Västerbotten had to be lower to avoid overlap because fewer individuals in total were available to sample from. In a second step I also sampled males and females separately (Dalarna-Gävleborg, n = 50; Västerbotten, n = 30) to investigate if there are detectable differences between mother-daughter and father-son dyads.

To test for differences in the completeness of the pedigree I used Pearson's Chi-square test for homogeneity of proportions with the proportions corresponding to the number of parent-offspring pairs in the pedigree per number of sampled individuals.

To further test whether there is a spatial effect on parent-offspring pairs in sex-separated pedigrees, I also sampled males and females randomly across the whole sampling area (Dalarna-Gävleborg, n = 50; Västerbotten, n = 30).

3.3.4.2 Movement and reproductive success

When at least two sampling locations of the same individual were known, I used ArcMap to measure the Euclidian distance. In case of multiple locations the maximum distance was measured. I tested for differences in travel distances between males and females in both sampling areas using one-way ANOVA followed by a *post hoc* Tukey-Kramer test in JMP Pro.

The frequency at which an individual occurs in the pedigree can be seen as a measure of reproductive success. I used simple linear regression to predict frequency in the pedigree based on distance travelled.

3.3.4.3 Spatial Clusters

I used Optimized Hot Spot Analysis with the Fishnet Polygon option selected in ArcMap to search for population clusters based solely on spatial data (median centers of individuals). The method reveals areas with significantly higher clustering (hot spots) as well significantly lower clustering (cold spots) compared to what could be expected from a random distribution of the analyzed features (locations of individual bears in this study). I used the results to search for regions with high concentrations of bears, especially putative female core areas.

4 Results

The 2012 consensus genotype dataset for Dalarna-Gävleborg contained 434 genotypes; 244 females and 190 males. The female to male ratio was 1.28 which is significantly different from 1:1 ($\chi^2(1) = 6.72$, $p = .010$).

From the 2014 Västerbotten survey, 677 fecal samples could be linked to 270 individual bears. Five of those were excluded from subsequent analyses due to inconclusive sex. The consensus genotype dataset therefore contained 265 individuals; 136 females and 129 males. The female to male sex ratio of 1.05 did not differ significantly from 1:1 ($\chi^2(1) = 0.19$, $p = .667$).

The typing error was estimated as 1.538×10^{-4} for Dalarna-Gävleborg and 0.01 for Västerbotten.

4.1 Pedigree reconstruction

The FRANz (Riester et al. 2009) reconstructed pedigrees can be found in Appendix I (Dalarna-Gävleborg) and Appendix II (Västerbotten).

As shown in Figure 5, the MCMC algorithms used by FRANz to construct ML pedigrees do not appear to be prone to noise. The software consistently produced nearly identical results for assigned parentage and full-sibling (FS) relationship across multiple runs of the same data at three different sampling intensities (Fig. 5a). Although higher sampling intensities (= more genotypes) produce larger numbers of parent-offspring pairs in the resulting pedigrees, the variation in the results from multiple runs is practically the same at all three tested sampling intensities (Fig. 5b) with the exception of the number of putative full-siblings (No. FS). However, the FS assignment is currently still an experimental feature in FRANz (Riester et al. 2009).

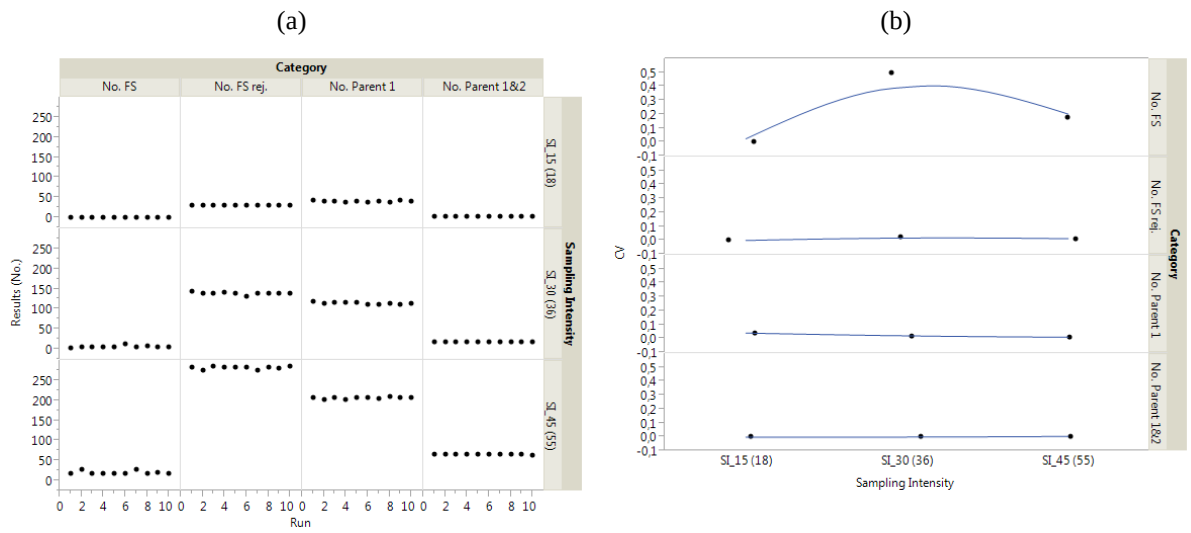


Figure 5 Test results for intrinsic noise in the FRANz pedigree reconstruction software. "SI" stands for sampling intensity and "No." for number of identified full-siblings (FS), parent-offspring dyads (Parent 1) and parent-offspring triads (Parent 1&2). The estimates of key parameters (parentage and full-sibling assignment) remain constant across multiple runs (a). The variation of results from multiple runs, plotted as the coefficient of variation (CV) on the y-axis in (b), is practically the same at different sampling intensities except for the experimental full-sibling (No. FS) feature. These results indicate that the ML pedigrees produced by FRANz remain unbiased by changes in sample size.

Figure 6 shows that incorrect settings of the typing error can strongly affect the resulting pedigrees. If the typing error setting is higher than the true value, FRANz will construct many false parent-offspring pairs. Conversely, typing error settings which are much below the true value could result in an empty pedigree.

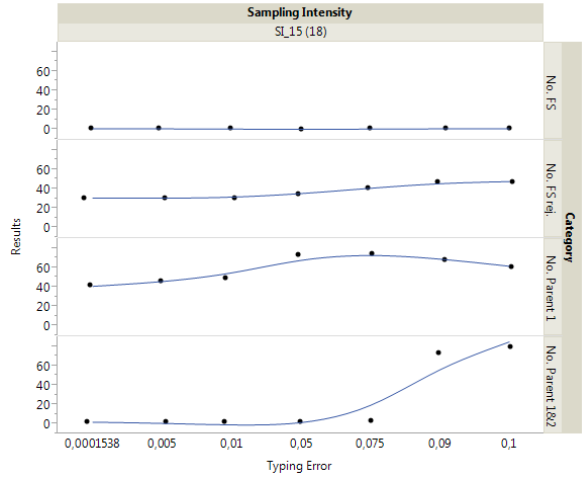


Figure 6 The effect of incorrect typing error (TE) settings on the ML pedigrees constructed by FRANz. In this example the true TE is 1.538×10^{-4}. Higher settings results in an increasing number of false parent-offspring dyads (No. Parent 1) and triads (No. Parents 1&2) in the pedigree.

4.2 Population estimates

4.2.1 Rarefaction analysis

For Dalarna-Gävleborg the rarefaction analysis was based on 873 samples containing 404 genotypes and 677 samples encompassing 270 genotypes for Västerbotten respectively. Figure 7 shows the estimated population sizes suggested by the amplitudes *a* of the

extrapolation curves constructed from three different models. Table 1 provides a summary of the model parameters and comparisons, indicating that the model suggested by Kohn et al. (1999) is the best fit for the data from both Dalarna-Gävleborg and Västerbotten. The resulting population estimates are

$\widehat{N}$ = 957 (Dalarna-Gävleborg)

$\widehat{N}$ = 488 (Västerbotten).

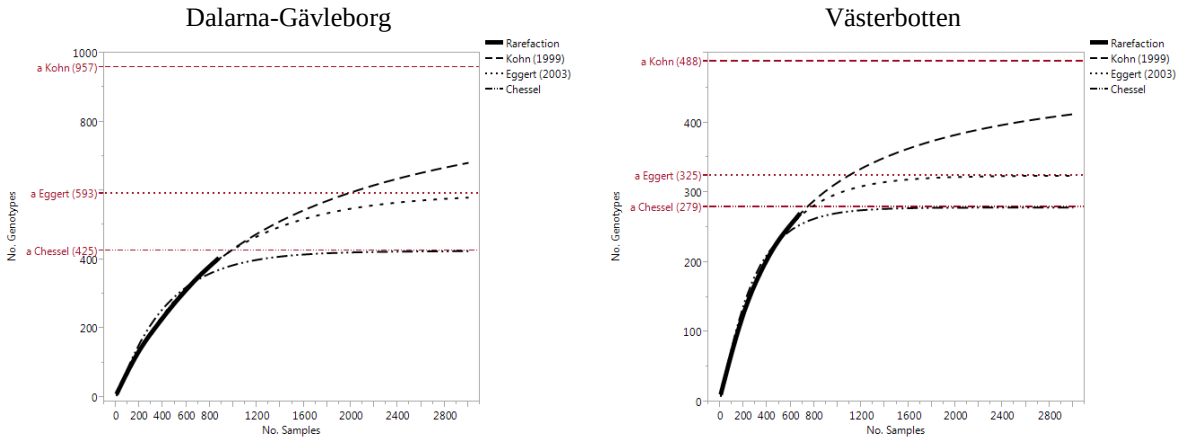


Figure 7 Rarefaction analysis results for Dalarna-Gävleborg (a) and Västerbotten (b). The bold black line represents the rarefaction curve based on the fecal samples collected in both counties. The dashed lines are the extrapolations by the three models fitted to the data. The values for the asymptotes *a* (= estimated population size) for each model are denoted by the dashed lines in red.

Table 1 Comparison of the three rarefaction analysis models. As indicated by the highest R^2 and lowest values for root average square error (RASE) and average absolute error (AAE), the model suggested by Kohn (1999) provides the best fit to the rarefaction curve data. The amplitude values (*a*) correspond to the estimated population sizes.

	Dalarna-Gävleborg					**Västerbotten**				
	Parameters		**Measures of fit**			**Parameters**		**Measures of fit**		
	a	*b*	*R²*	*RASE*	*AAE*	*a*	*b*	*R²*	*RASE*	*AAE*
Kohn (1999)	957.37	1213.56	0.999	4.32	3.72	488.06	548.09	0.999	1.80	1.44
Eggert (2003)	592.63	-0.0013	0.998	5.10	4.37	324.57	-0.0025	0.999	2.28	1.99
Chessel	425.22	n.a.	0.971	18.95	16.64	278.80	n.a.	0.985	9.18	7.98

4.2.2 Pedigree reconstruction method (Creel-Rosenblatt estimator)

Figure 8 depicts the results for the population size estimates based on the Creel-Rosenblatt estimator and rarefaction analysis in comparison to the official county estimates that were calculated using the closed population model Mth2 in the software MARK (White and Burnham 1999) and the large carnivore observation index (LCOI) (Jonas Kindberg, pers. comm.). For the Dalarna-Gävleborg bear population the CRE estimate is $\widehat{N}$ = 623 with a lower bound of 434 (= count of genotypes) and an upper limit of 731(assuming no mortality in the maximum number of individuals that could be inferred from the pedigree). The rarefaction analysis suggested $\widehat{N}$ = 957 individuals, 95% CI [947, 967].
For Västerbotten the results are $\widehat{N}$ = 404 (lower bound: 265, upper bound: 476) for the CRE and $\widehat{N}$ = 488, 95% CI [486, 489] for the rarefaction.

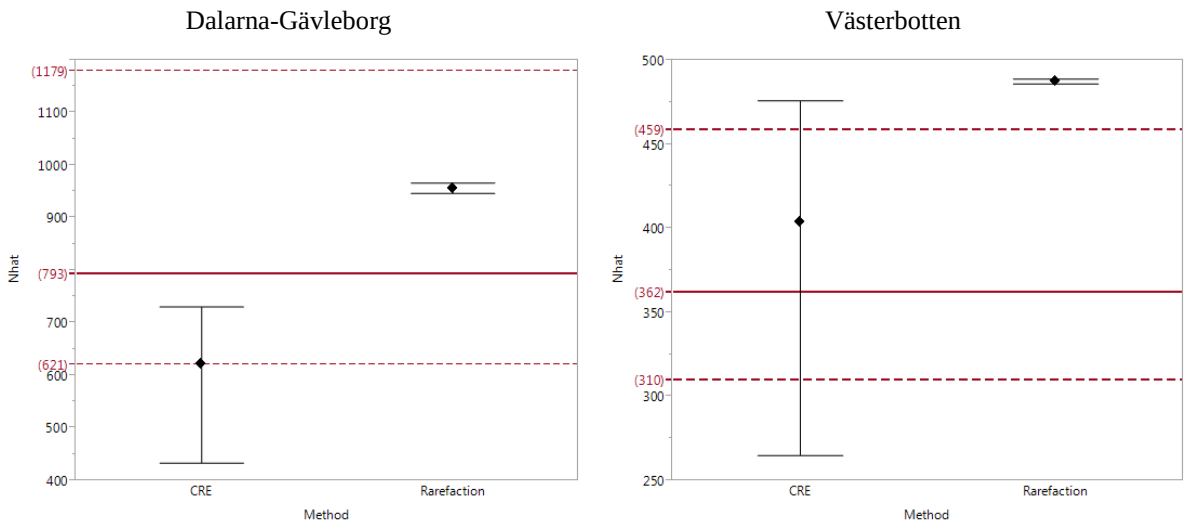


Figure 8 Population estimates by the Creel-Rosenblatt estimator (CRE) and from rarefaction analysis. The bold red lines correspond to the official population estimates and their 95% confidence interval (dashed red lines). The error bars on the CRE estimates do *not* denote confidence limits. Instead, the lower bound represents the genotype count and the upper bound the maximum estimate based on the assumptions of no mortality and no mating with multiple partners among the inferred individuals. In the rarefaction results the error bars represent 95% bootstrap confidence limits. In both counties the CRE population size results fall within the 95% confidence limits of the official estimations. The rarefaction analysis based on Kohn's (1999) model appears to have a tendency to overestimate.

Table 2 lists the numbers of individual genotypes that were used at the different sampling intensities as well as the resulting population estimated based on the CRE and rarefaction. The performance of the two estimators in comparison to each other and to the official population estimates is shown in Fig. 9a and the model gain of the CRE over a simple count of directly sampled individuals in Fig. 9b.

Table 2 Bear population estimates ($\hat{N}$) for Dalarna-Gävleborg (DG) and Västerbotten (V) based on the Creel-Rosenblatt estimator (CRE) and rarefaction analysis (Rf) at different sampling intensities (SI). The bold values for SI correspond to the percentage that the number of individuals in the sample (*n*) represents compared to the estimated total population size based on rarefaction analysis. The two numbers following in parentheses are the percentages which *n* represents of the official population estimates for the counties (first value DG, second value V). For example: The initial rarefaction estimate for the DG was 957. A 15% SI corresponds to *n* = 144. The official population estimate, however, is 793 of which a sample size of *n* = 144 represents 18%.

	Sampling intensity (SI)											
	15% (18, 20)			**30%** (36, 40)			**45%** (55, 60)			**54%** (n.a., 73)		
	n	$\hat{N}$		*n*	$\hat{N}$		*n*	$\hat{N}$		*n*	$\hat{N}$	
		CRE	*Rf*		*CRE*	*Rf*		*CRE*	*Rf*		*CRE*	*Rf*
DG	144	193 (144, 204)	489 [475, 503]	287	409 (287, 474)	733 [717, 750]	434	623 (434, 731)	957 [947, 967]	n.a.	n.a.	n.a.
V	73	93 (73, 101)	192 [179, 203]	146	211 (146, 234)	419 [406, 433]	219	318 (219, 388)	493 [486, 499]	265	404 (265, 476)	488 [486, 489]

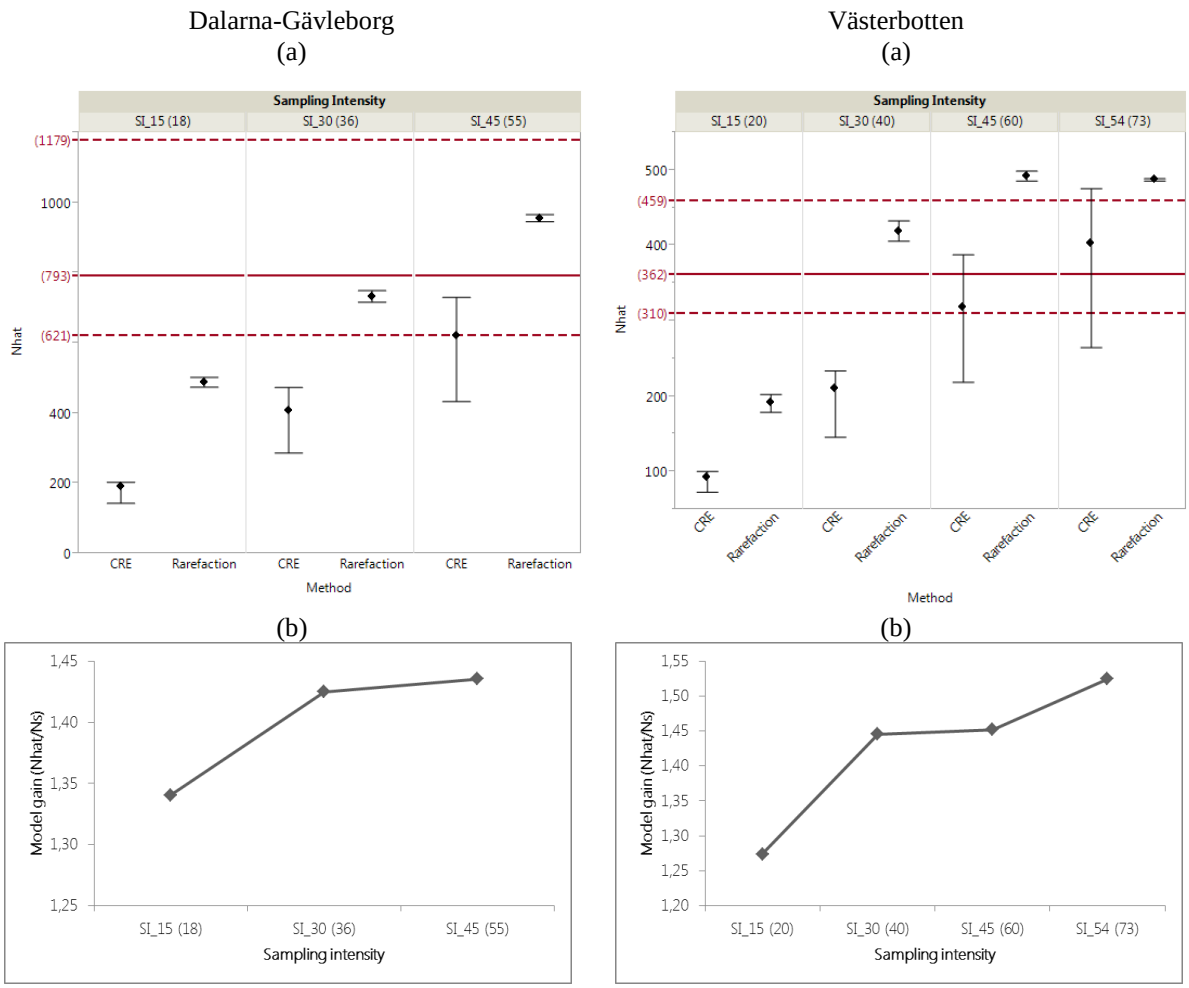


Figure 9 Comparison of the performance of the Creel-Rosenblatt estimator (CRE) and rarefaction analysis at different sampling intensities (a) and the gains of the CRE relative to a count of sampled individuals *N*s (b).

4.3 Genetic characteristics

4.3.1 Genetic diversity, inbreeding and gene flow

For the Dalarna-Gävleborg population Fahlen (2014) reported HWE, an average F_{is} of -0.0014 and male-mediated gene flow from 7 migrants of the eastern lineage.

In Västerbotten mean H_o (0.431) was lower than H_e (0.448). The initial analysis across all 85 autosomal SNPs showed 13 loci out of HWE and an average F_{is} of 0.035. Eight loci that displayed a highly significant ($p < .001$) deviation from HWE were removed. After removal, deviation from HWE was no longer significant, $\chi^2(154) = 167.02$, $p = .22$. The corresponding mean inbreeding coefficient was slightly lower at $F_{is} = 0.025$ but still significantly different from zero, $t(76) = 3.21$, $p = .002$.

I found one male migrant (AC2014-081) with the southern haplotype (western lineage). The pedigree indicated that the individual had bred with at least four females. One of his male offspring had in turn also already produced offspring (Fig. 10).

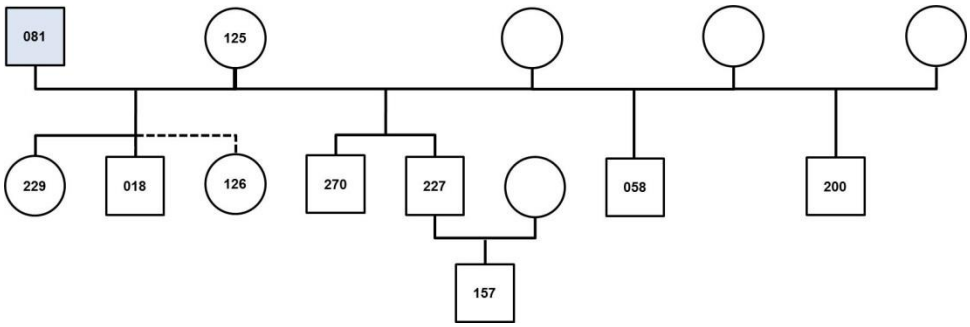


Figure 10 Known relationships of a male migrant (081) from the western lineage (southern haplotype) in Västerbotten. The putative female offspring 126 is connected with a dashed line because 081 and 125 were identified as parents in the pedigree but no full-sibling relationship with 018 and 229 was found by the 'fullsibtest' experimental feature in FRANz. Empty circles represent unsampled females that were inferred by pedigree reconstruction.

4.3.2 Population structure

For Dalarna-Gävleborg Fahlen (2014) reported $K = 1$ genetic clusters based on the LnP(D) method and the PCA. The *ad hoc* statistic ΔK suggested three sub-populations ($K = 3$).
In Västerbotten LnP(D) appeared a bit inconclusive (see discussion) whereas ΔK suggested two subpopulations. The PCA resulted in one diffuse cluster and the GENELAND results based on spatio-genetic data indicated four clusters (Fig. 11 & 12).

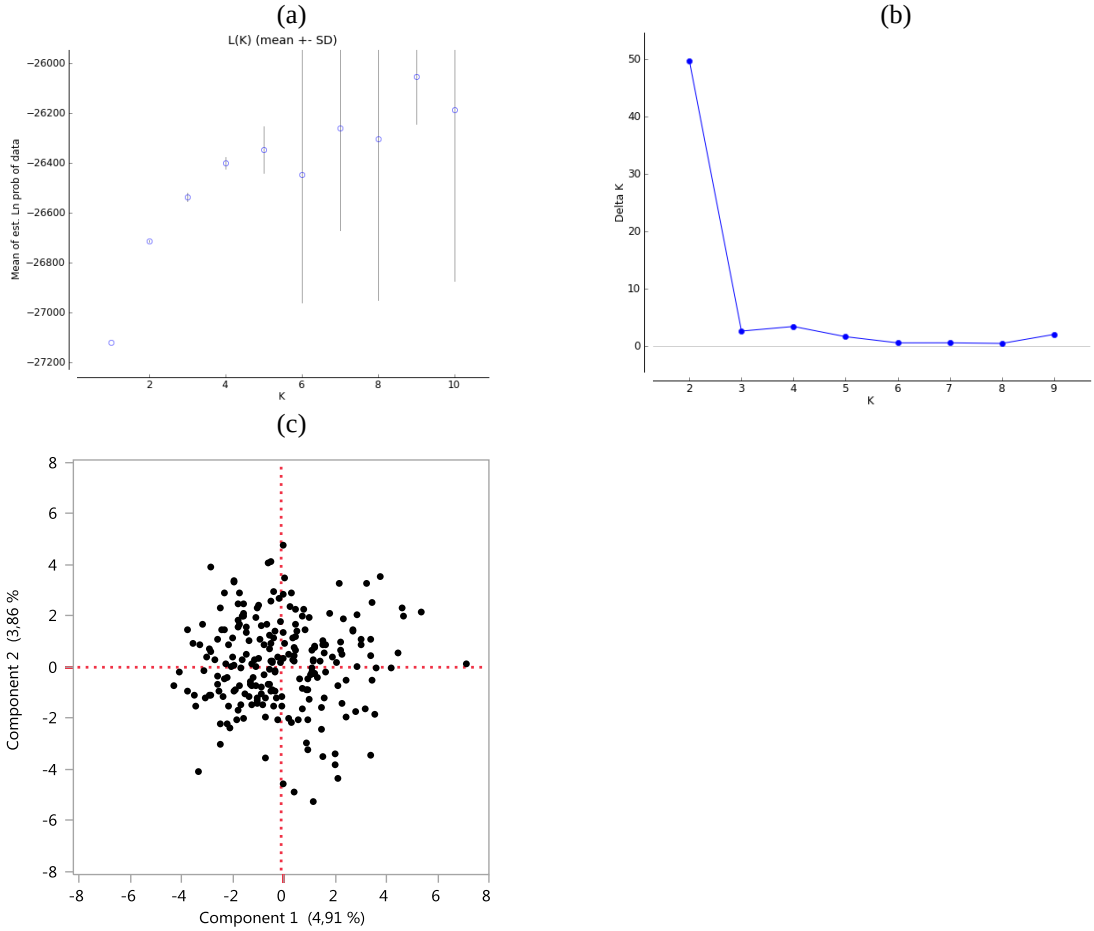


Figure 11 Suggested number of sub-populations (clusters) K in Västerbotten according to different methods; (a) LnP(D), (b) ΔK and (c) PCA.

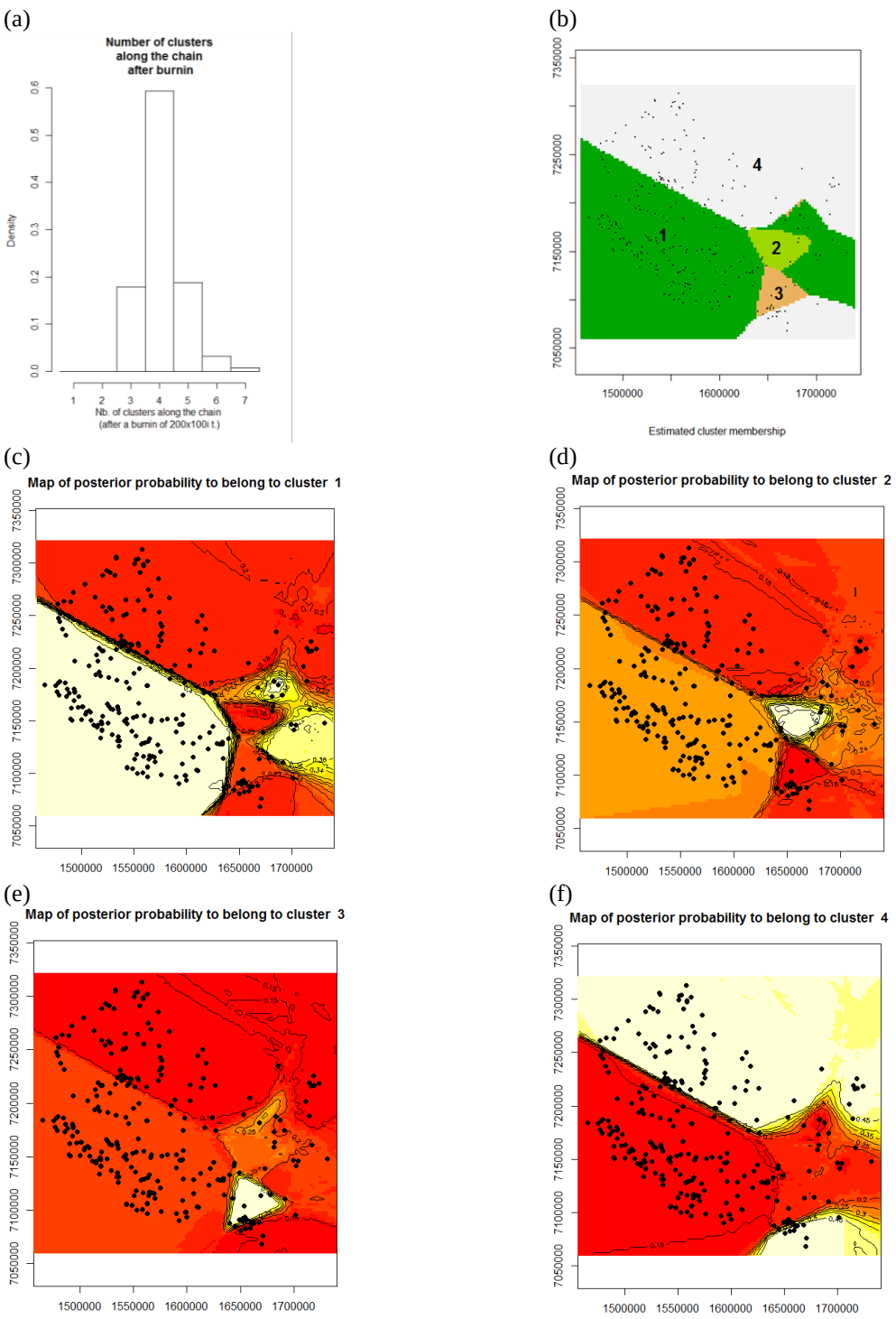


Figure 12 The GENELAND analysis suggests four (a) spatio-genetically distinct population clusters (b). The maps (c-f) depict the posterior probabilities of individuals (black dots) belonging to a particular cluster on a white-to-red graded scale with white representing the highest values.

The comparison of the clusters suggested by GENELAND to major roads as a possible explanation for population fragmentation (see Appendix III), revealed no clearly discernable pattern.

4.4 Spatial investigations

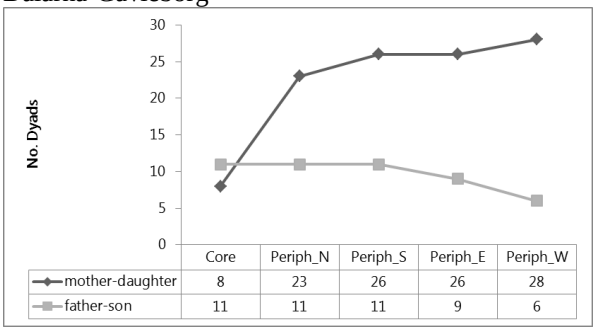
4.4.1 The effect of borders on the pedigree

I found no significance differences in the completeness of the pedigree between the core area and the four peripheral border areas in Dalarna-Gävleborg, $\chi^2(4) = 7.05$, $p = .134$ or Västerbotten, $\chi^2(4) = 1.97$, $p = .74$.

When males and females were sampled separately, significantly more mother-daughter than father-son dyads were found in Dalarna-Gävleborg, $\chi^2(9) = 62.79$, $p < .0001$. When the same number of males and females (n = 50) were sampled randomly across the whole area there was no significant difference between the proportions of mother-daughter and father-son dyads per sampled individuals, $z = -0.521$, $p = .602$.

In Västerbotten, visual inspection of the data (Fig. 13) suggests the same trend but the difference in proportions between male and female dyads was not significant, $\chi^2(9) = 15.28$, $p = .083$. The two-sample *z*-test for proportions when females and males (n = 30) were sampled randomly across the whole area was also not significant, $z = -0.645$, $p = .52$.

Dalarna-Gävleborg



Västerbotten

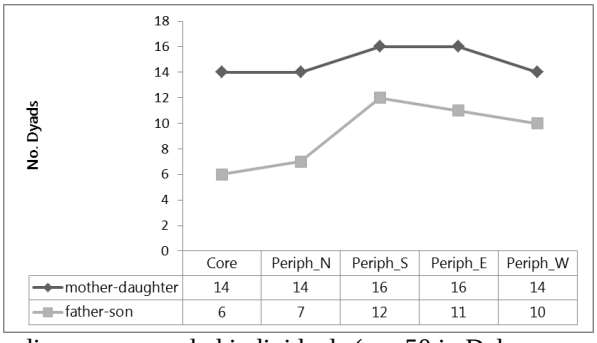


Figure 13 Mother-daughter and father-son dyads in the pedigree per sampled individuals (n = 50 in Dalarna-Gävleborg, n = 30 in Västerbotten) in the core and along the peripheral boundaries of the counties. Except for the core of Dalarna-Gävleborg there are generally more mother-daughter than father-son dyads in each area although results for Västerbotten are not statistically significant.

4.4.2 Movement and reproductive success

In Dalarna-Gävleborg the movement distances inferred from multiple fecal sites for males (n = 62) averaged $M = 29.0$ km, $SD = 31.4$ km. The median was 17.0 km, the maximum 204.0 km and the minimum 0.05 km. For females (n = 70) the mean was $M = 26.9$ km, $SD = 33.7$ km. Median distance was 16.4 km, the maximum 177.2 km and the minimum 0.6 km.

In Västerbotten males (n = 65) moved on average $M = 28.1$ km, $SD = 34.7$ km with the median being 15.3 km and the minimum / maximum distances 188.6 km and 0.4 km respectively. The numbers for females (n = 65) are $M = 11.1$ km, $SD = 8.2$ km, median = 8.6 km, max = 33.6 km and min = 0.6 km.

One-way ANOVA for testing difference in average movement was significant, $F(3, 258) = 5.50$, $p = .001$. The *post hoc* Tukey-Kramer test indicated that females in Västerbotten ($M = 11.1$ km, $SD = 8.2$ km) travelled significantly shorter distances than the other groups (males and females in Dalarna-Gävleborg, males in Västerbotten).

The regression of 'frequency in the pedigree' on 'travel distance' was significant in Dalarna-Gävleborg, $F(1, 30) = 4.18$, $p = .043$, $R^2 = .031$ (Fig. 14). No significant correlation was found in Västerbotten, $r = -0.094$, 95% CI [-0.26, 0.08], $p = .29$.

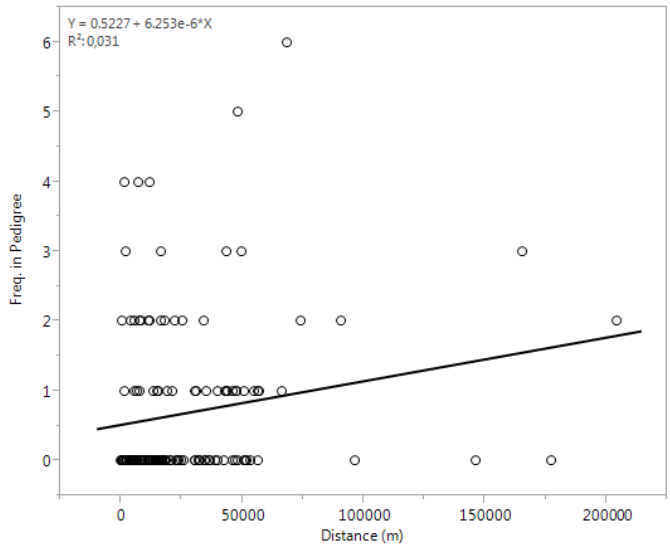


Figure 14 Regression of frequency in the pedigree on distance travelled by bears in Dalarna-Gävleborg. The positive correlation suggests that larger movements result in more offspring. However, the effect is very weak as indicated by the low coefficient of determination (R^2), explaining only approximately 3% of the variation.

4.4.3 Spatial clusters

The Optimized Hot Spot Analysis detected areas with significantly higher and lower spatial clustering of female and male individuals in both counties (Fig. 15).

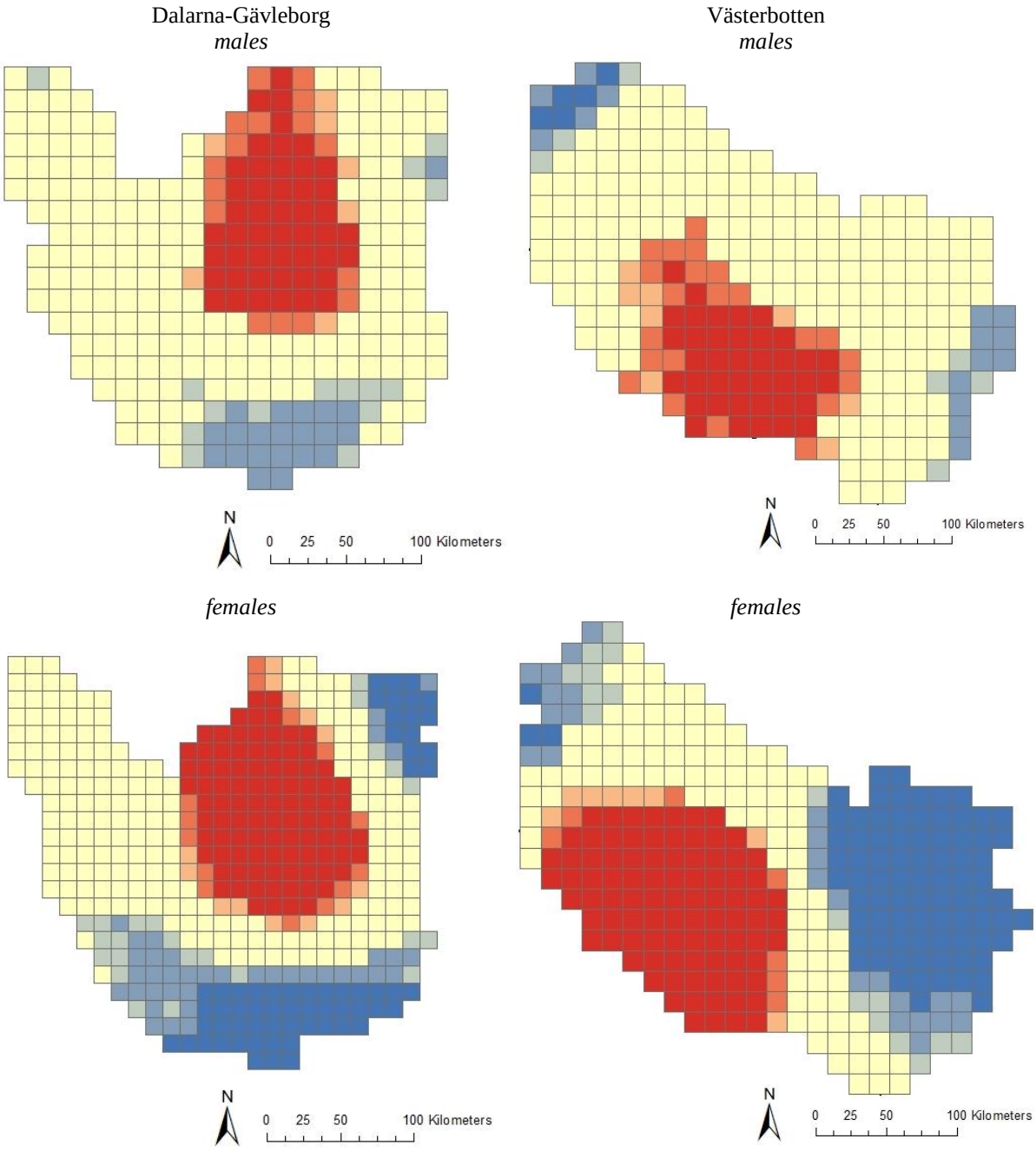


Figure 15 Optimized Hot Spot Analysis clusters of males and females in Dalarna-Gävleborg and Västerbotten. Red areas (hot spots) indicate significantly higher clustering of individuals than could be expected from random distribution of all sampled individuals and blue areas (cold spots) represent the opposite (significantly less clustering). While the hot spots could to some degree be due to higher sampling effort in these areas, the much larger cold spots in the female distribution in both counties suggest that females really are more concentrated in the hot spot areas. This could be an indication of female core areas.

5 Discussion

In this study, I used the Creel-Rosenblatt estimator (CRE) as a new, pedigree reconstruction-based method to estimate the size of two Swedish brown bear populations. SNP genetic data obtained from non-invasive fecal samples was used in the reconstruction of pedigrees and for investigations into population structure, genetic health and spatial patterns. With regard to my hypotheses I summarize:

(i) Total population size could be estimated with the CRE using pedigree reconstruction on the basis of SNP genotypes with 85 autosomal loci. The performance of the estimator on empirical genetic data was similar to the results of simulations by Creel and Rosenblatt (2013).

(ii) If the sampling intensity is ≥ 40% the CRE provides results that are comparable to rarefaction analysis and also to capture-mark-recapture methods; at lower sampling intensities the CRE tends to underestimate. The gain in population size estimates relative to a count of genotypes ranged from 27-52%.

(iii) An inbreeding coefficient of $F_{is} = 0.025$ could indicate low levels of inbreeding in Västerbotten. However, the value is not high enough to consider the population genetically unhealthy.

(iv) One male migrant from the western lineage (southern haplotype) was found.

(v) The use of spatial data extended the range of possible tests for population structuring. Female core areas suggested by clusters inferred from purely spatial data corresponded with the findings of others. While more prone to bias and error than observational data, inferred movements from fecal site locations appear to be a viable source for spatial analyses.

(vi) There was no significant difference in the completeness of pedigrees from samples collected at different parts (core and peripheries) of the study area.

(vii) There was no strong evidence for linking movements to reproductive success.

Pedigree reconstruction

The sampling effort by citizen volunteers across the study area was high enough to allow for the construction of rarefaction curves for an initial approximation of the population size which was needed for the parametrization of the FRANz pedigree reconstruction software. If the sampling effort is too low, the rarefaction graph would not start to converge asymptotically but remain linear (with every collected sample corresponding to a new genotype) and no population estimate could be made. In such a situation and in the absence of prior population estimates, heuristics based on general knowledge about the species could be used. For example, if approximate home range sizes are known, the maximum population size could be estimated as the number of potential home ranges in an area. Correct settings of the typing error are of particular importance. FRANz constructs maximum likelihood pedigrees largely based on genetic similarity between genotypes. A common exclusion criterion for putative parent-offspring dyads is the presence of opposing homozygotes. For example, if an assumed parent is homozygous at a particular locus (e.g. TT) then its offspring cannot be homozygous for a different allele (e.g. AA) at that same locus (Calus et al. 2011). However, it is possible that an identified homozygous locus resulted from a common error like misprinting or allelic dropout during the genotyping process. In such a case, the pedigree reconstruction software needs the typing error as a measure of how many such instances it can reasonably expect and tolerate. If the error rate

is set below its true value, the software will reject many otherwise perfectly fine parent-offspring matches based on a few erroneously genotyped loci. In the extreme, this could result in an empty pedigree. Conversely, if the error rate is set too high, the software will ignore too many true exclusion scenarios and establish relationship links between individuals who are in reality unrelated (Wang and Santure 2009).
The implications of neglecting to address the typing error in genetic data can be dramatic. In a study on wolves in Yellowstone National Park, Creel et al. (2003) showed that ignoring typing errors resulted in a 5.5 fold overestimation of population size. In the Yellowstone example, typing errors led to the so called "ghost effect" where one genotype is falsely interpreted as several different ones. Non-invasively collected samples, particularly feces, are especially prone to typing errors due to the often heavily degraded DNA and contamination by non-target DNA from bacteria and food. In a later paper, Creel and Rosenblatt (2013) point out that "ironically the ghost effect becomes stronger if the number of loci is increased to eliminate the shadow effect", with the latter being the misidentification of several individuals as one.
A common way to estimate the typing error and to obtain reliable genotypes is to perform several genotyping runs on the same sample (Hess et al. 2012) in a so-called multiple tube approach (Taberlet et al. 1999). Alternatively, FRANz offers the option to specify known parent-offspring relationships *a priori* and the software then estimates the error rate automatically from these pairs (Riester et al. 2009). However, no prior information about relatedness of the sampled bears was available for this study.

Population estimates
A useful measurement for precision is the percentage relative precision (PRP) which relates a population estimate to its 95% confidence limits (Sutherland 2006). The PRP of the official bear population estimates was 21% in Västerbotten and 35% in Dalarna-Gävleborg. The circumstance that precise population estimates were available prior to this study provided an ideal framework for testing a new census method because it allowed for verification of the results.
The pedigree reconstruction-based population estimates of the CRE fell within the confidence limits of the official numbers. This is an indication that the method provides a viable alternative to CMR approaches with the added benefit that it can even be employed to data from a single sampling event. The CRE increased the number of detected individuals by 27-52% compared to a simple count. However, as already demonstrated by Creel and Rosenblatt (2013) in their simulations, the method works best if the sampling intensity exceeds ~40%. At lower sampling intensities, the estimator tends to severely underestimate the true population size and also underperforms in comparison with rarefaction analysis. This can be explained by the fact that small sample sizes usually do not contain many parent-offspring pairs which severely restricts the pedigree reconstruction. At much higher sampling intensities (e.g. >80%) hardly any information is gained over a simple count. Moreover, the risk of overestimation also increases. If, to give an extreme example, 100% of individuals were sampled and no reliable information of mortalities was available, the CRE would severely overestimate the size of the population because individuals (albeit dead) would still be inferred from the pedigree. This situation can be avoided by comparing the CRE estimates to those obtained from rarefaction analysis on the same data. I recommend to always consider all three suggested rarefaction models. The model which best fits the data based on R^2 and the error readings, should be treated as the most informative one but the others can still provide useful auxiliary information. For example, in situations when sampling intensity is high, the model suggested by Kohn et al.

(1999) tends to overestimate while Chessel's model (Petit and Valiere 2006) is more likely to underestimate the population size. Thus, the best population estimate probably lies in between.

In their simulations, Creel and Rosenblatt (2013) tracked all individuals throughout the simulated period of 15 years which means that they consistently had accurate information about parent-offspring relationships and mortalities. Based on these data, they were able to infer individuals (as the missing parent in parent-offspring dyads) without error. In pedigrees reconstructed from empirical genetic field data, this inference is less straightforward for species with multiple mating behaviors. The authors already address this matter by recommending a refinement of their model to account for mating behavior and population subdivision.

In the pedigrees that were reconstructed for this study, inferring the missing parent in each dyad as a new individual would most likely lead to an overestimation because male bears mate with multiple females and vice versa (Steyaert et al. 2012). As pointed out earlier, I therefore used this assumption only for calculating the upper limits of the CRE population estimates. Naturally, each offspring can only have one mother and one father. However, if there are, for example, five mother-offspring dyads in the pedigree, the number of inferred fathers could be anywhere between one and five. Using the ratio of known dams and sires for inference of individuals remains somewhat unsatisfactory because it probably does not fully reflect reality. However, for lack of a better method given the data, it should suffice and prevent overestimation.

A preferable approach would be to start from the maximum inference (N_{in} = number of dyads) and then account for multiple parentages based on the relatedness of the offspring. For example, offspring with the same parents should be detectable as full-siblings. FRANz currently offers an experimental feature (--fullsibtest) to identify full-siblings. However, in almost all instances the detected full-siblings corresponded to parent-offspring triads in the pedigree. From these constellations no further individuals could be inferred because in parent-offspring triads there is no "missing" parent. Additionally, I found instances in which two individuals shared the same parents according to the pedigree but were not detected as full-siblings by the --fullsibtest routine. For many of the reconstructed parent-offspring dyads I suspected offspring to be half-siblings, individuals who share one parent. If, for instance, in the above example all offspring in the five mother-offspring dyads were half-siblings, we could then infer the presence of only one individual (their common father). Relatedness between all sampled individuals could be calculated as the Lynch-Ritland relatedness coefficient (Lynch and Ritland 1999). However, the coefficient only captures the degree of relatedness and not the specific relationship. Half-siblings share on average approximately 25% of alleles but the same is true for grandparent-grandoffspring and avuncular relationships (Blouin 2003). Thus, the true relationship between two individuals can usually not be inferred from their degree of relatedness alone.

In order to really improve inferences from the pedigree, information about the age of the sampled individuals is needed. If genetic relatedness can be combined with age in the analysis, the most probable relationships can easily be determined.

I recommend keeping track of each genotyped individual from the date it was first recorded. Even if the true age remains unknown, a minimum age can be assigned and over the course of several sampling periods, individuals can at least be compared on the basis of age relative to one another. This would considerably improve the accuracy of the CRE population estimates.

Another current weakness of the CRE lies in the difficulty to assign confidence limits. Further research is needed to develop appropriate methods. Bayesian simulations or bootstrapping might be viable options.
Furthermore, the CRE is only suitable for non-cyclical, long-lived species with either low or well documented mortality rates due to the possible bias arising from inferring individuals which might be dead by the time their existence is concluded from the pedigree (Creel and Rosenblatt 2013). For the Swedish bear population individual mortalities would most likely be detected because natural mortality of adults is minute in comparison to hunting or traffic accidents which are recorded (Morner et al. 2005).
Sometimes population studies are faced with the problem that juveniles tend to be missed in the sampling because they are hiding in a den or otherwise sheltered area (Logan 2001, Ross and Jalkotzy 1992). However, this is not applicable to bears because cubs start following their mothers around as soon as they emerge from their dens after hibernation.
For well-studied populations that are regularly sampled, the CRE offers no immediate advantage over established CMR methods in terms of estimating population size. However, if sampling is repeated over a number of years, the required sampling effort to maintain a desired sampling coverage is strongly reduced as genotyped individuals accumulate. In simulations by Creel and Rosenblatt (2013) the proportion of the population that had to be sampled typically dropped to ≤ 20% within three years (Fig. 16).

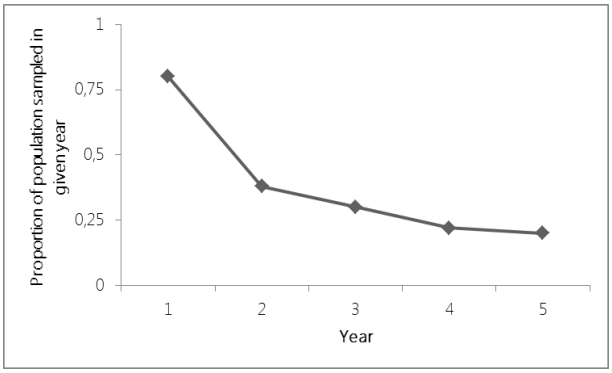


Figure 16 The proportion of the population that has to be sampled in in each given year to maintain a desired sampling coverage (here, approximately 75%) strongly declines over time if the monitored species is long-lived and has a low turnover rate. The graph is based on results published by Creel and Rosenblatt (2013).

If the results of the simulations are verified by empirical studies, the CRE has the potential to outperform CMR in terms of the required sampling effort, time and cost. In the meantime, efforts should be undertaken to improve established CMR methods by incorporating individuals inferred by pedigree reconstruction into the calculations.
The CRE could also prove to be very useful in situations where budgetary or logistic restraints make repeated, large scale sampling events unrealistic; for example in remote regions or developing countries. The use of non-invasively collected samples like feces could aid in making studies under such adverse conditions feasible. The identification, collection and transport of target feces require little training, which is advantageous in the recruitment of citizen volunteers. The negative trade-offs in terms of possible contamination and degradation can be alleviated to some degree by using SNPs as the molecular marker of choice. Because of their reproducibility across laboratories, the genotyping work and costs could also be shared between cooperating research institutions.

Genetic characteristics

The significant departure from Hardy-Weinberg equilibrium was largely due to 8 of the 85 autosomal loci. Further study is needed to determine the cause of the deviation from Hardy-Weinberg proportion at these loci. Possible explanations include genotyping errors and selection (Waples 2015). An indication that selection pressure really might be at work is the circumstance that the average F_{is} value of 0.035 for all 85 loci corresponds well with findings of Schregel et al. (2012) who reported an F_{is} of 0.04 for bears from Västerbotten. However, their study used short tandem repeats (STRs) rather than SNPs as genetic markers so the results might not be directly comparable. Until the question is resolved, the F_{is} of 0.025, which was based on an analysis that excluded the 8 questionable loci, should be treated as the more probable value for the inbreeding coefficient. The positive F_{is} indicates heterozygote deficiency which can suggest inbreeding. However, the number is close to zero and presently need not raise serious concern about the genetic health of the Västerbotten population. To provide a comparison, Liberg et al. (2005) reported inbreeding coefficients one order of magnitude higher (≥ 0.25) for a Scandinavian wolf (*Canis lupus*) population that showed clear signs of inbreeding depression like reduced litter sizes and the resulting decline of the population growth rate (λ).

Fahlen (2014) reported male-mediated gene flow from the eastern lineage into the southern Swedish bear population and points out the current paucity of data on the reproductive success of migrants. This study found one male migrant with the southern haplotype in Västerbotten that had produced offspring with four females.

Population structure

Researchers who use the software package STRUCTURE often infer the number of genetic clusters *K* from the highest LnP(D) which is the log likelihood for each step in the MCMC based Bayesian algorithm the software uses to estimate the number of clusters (Pritchard et al. 2000). This approach is frequently not very conclusive because multiple runs for each *K* often show significant overlap of the LnP(D) values (Fig. 11a). A more reliable method is the one based on ΔK as proposed by Evanno et al. (2005) which suggested two clusters (or sub-populations) for Västerbotten. It should be noted, however, that the method assumes that there really is population sub-structuring present. In other words, values of $K = 1$ cannot be detected. In a recent paper, Meirmans (2015) cautions against relying too much on the "optimal" *K* values suggested by STRUCTURE and recommends to consider only clusters with a clear biological explanation. The GENELAND analysis (Fig. 12), which was based on the combination of spatial and genetic data, suggested four clusters. This result is also not fully convincing because the posterior probabilities for individuals belonging to a particular cluster are mostly around 0.4 which is rather low. However, similar values were deemed sufficient for the inference of population clusters in a study by Rieux et al. (2011). It is interesting that the majority of individuals in the GENELAND results are assigned to two clusters (1 and 4) with clusters 2 and 3 encompassing only tiny proportions. This could be viewed as further evidence of there being two sub-populations in Västerbotten as suggested by the ΔK results. Moreover, the spatial division between cluster 1 and 4 appears to coincide with county Highway E12 (see Appendix III). It is possible that the E12 presents an obstacle that fragments the population and restricts gene flow although no such effect was found in connection with other highways of similar size. However, Highway E12 also runs mainly along the side of the Umeälven and a chain of major lakes which might reinforce the fragmenting characteristics. Further studies of landscape genetics (Manel et al. 2013) are needed for clarification. Until then the results should be viewed as speculative.

The PCA result (Fig. 11c) suggests only one cluster although a very slight division can be seen at the center of the score plot. However, the individuals within these putative "clusters" did not conclusively match the individuals in clusters 1 and 4 of the GENELAND analysis but were distributed seemingly random over the whole study area. Although SNPs generally have sufficient power to detect population structure (Mesnick et al. 2011), the SNP panel used in this study was designed with primary focus on relationships and might therefore not be fully informative for population structure. While I found indication of at least two sub-populations in Västerbotten the results are not conclusive and it cannot be ruled out that no such genetic structuring exists. Further studies could investigate whether the putative two sub-populations suggested by the analysis of nuclear DNA correspond with the two known mtDNA haplotypes (North A and North B) in Västerbotten.

Spatial investigations

Contrary to my hypothesis I found no significant differences in the completeness of the pedigree when sampling individuals from the peripheries of the study area compared to the center regions. This suggests that many individuals roam widely with frequent crossings in and out of the study area.

More mother-daughter than father-son dyads were found when sampling the same number of male and female individuals in specific areas. This may be explained by the often reported female philopatry in brown bears (Blanchard and Knight 1991, Saarma and Kojola 2007, Støen et al. 2005). Related females tend to stay together in overlapping home ranges (Støen et al. 2006) and are slow to colonize new areas (Glenn and Miller 1980). Additionally, disturbance along the peripheral regions of female core areas, such as hunting, can easily prevent female expansion (Swenson et al. 1994).

When comparing the known maximum movement distances between males and females from both counties, the females from Västerbotten stand out with on average significantly shorter movements. This observation appears to be not just the result of missing a few long-distance outliers because the median distance was also only approximately half of what was found for the other groups. It is important to bear in mind, that movement distances inferred from fecal sites can be strongly biased. For example, at least two samples have to be collected and successfully genotyped to infer a distance. It is possible that this simply was not the case for several far-roaming females in Västerbotten or perhaps they moved to areas where sampling intensity was low. Moreover, movement patterns also differ throughout the year. Huber and Roth (1993) reported similar daily movement distances for male and female bears in Croatia while on an annual basis, male ranges were up to five times larger than those of females. Especially during the mating season in spring, males roam widely in search of females (Krofel et al. 2010). Similarly, Dahle and Swenson (2003) found home ranges of males to be larger in Scandinavia but also observed clear seasonal patterns. During the berry season in the autumn, roaming is much reduced in both sexes once suitable feeding areas are found and the main focus is placed on gaining weight for the upcoming hibernation period. In marginal habitats, like northern Alaska, it has been observed that the search for food takes precedence even during mating season (Edwards and Derocher 2015). In both counties, the collection of fecal samples started in August but finished much later in Västerbotten (December) than in Dalarna-Gävleborg (October). It is possible, that many of the female samples in Västerbotten were collected at a time when movements were much reduced at the end of the berry season. That no significant differences in movement were found between males and females in Dalarna-Gävleborg corresponds well with the reports of similar behaviors during the berry season.

The slight positive correlation found between movement distances and frequency of occurrence in the pedigree as an indicator of reproductive success should be viewed as explorative and does not necessarily imply causation. The most obvious potential covariate I could not control for due to the absence of data is age. Obviously, the older an individual is the more chances it had to breed and will therefore more frequently appear as a parent in the pedigree. Moreover, older individuals might also move over larger distances. However, just because an individual moved longer distances during a particular sampling period does not necessarily mean that this is its general behavior. Nevertheless, the question of whether higher mobility of individuals results in more instances of mating remains an intriguing one and could only be answered through monitoring of actual movements over several mating periods. Pedigree reconstruction could then be used to evaluate whether individuals of similar age achieve different levels of reproductive success based on their mobility.
The clusters inferred from purely spatial data (Fig. 15) show distinct cold and hot spots for both males and females. Some of the effect is most likely due to higher sampling intensity in the hot spot areas because they coincide with reasonably accessible regions that are also much frequented by moose hunters. However, using only the median distances from several samples removed some of the possible bias resulting from variations in sampling effort. Moreover, in both counties the cold spots are much more pronounced for females. This lends support to the assumption that the female hot spots really are female core areas. The clusters are also a good match to areas of female concentration reported by Manel et al. (2004).

6 Conclusions

This study shows that SNP-based pedigree reconstruction can be used to estimate total population size for species with long generation times. This includes most large carnivores. The Creel-Rosenblatt estimator (CRE) can be applied to data from a single sampling event but should in such instances be supplemented with rarefaction analysis for comparison. The CRE shows great promise for use in longer term monitoring programs due to a rapid decline of the required sampling effort after the first intensive sampling event. However, further empirical studies are needed to confirm this. Future improvements of the method include devising a procedure to calculate confidence limits and to account for species-specific mating behavior. Incorporating age data into the pedigree reconstruction would much improve the accuracy of the estimates.
The Västerbotten bear population can be considered genetically healthy despite a slight indication of inbreeding, especially since there is evidence for male-mediated gene flow from the southern population. The degree of population sub-structuring remains at present inconclusive and should be further investigated, particularly with regard to the possible fragmenting effects of Highway E12.
I found indications that spatial data obtained from non-invasively collected fecal samples allows for inferences about movements if the limitations are clearly addressed. In combination with pedigree reconstruction, future studies could focus on dispersal patterns and reproductive success.

7 Acknowledgements

I thank my supervisors Göran Spong and Anita Norman for their invaluable support, advice and encouragement. Helena Königsson patiently explained lab procedures and the interpretation of Biomark genotype outputs. Joanna Fahlen's work on bears in Dalarna-Gävleborg provided valuable insights and guidance for my own research. Michael

Schneider from the County Administration Board kindly directed me towards current estimates of bear mortality rates and Jonas Kindberg from the Scandinavian Brown Bear Project emailed the official 2015 bear census results which were indispensable as a baseline for comparison.
Last but not least, credit is due to the numerous hunters and other volunteers who participated in the sample collections. Without their effort none of this work would have been possible.

8 References

Anderson, E. C. and Garza, J. C. (2006). The power of single-nucleotide polymorphisms for large-scale parentage inference. - Genetics 172: 2567.

Artdatenbanken. (2015). Björn (Ursus arctos). - SLU Artdatenbanken. http://artfakta.artdatabanken.se/taxon/100145.

Balme, G. A., Lindsey, P. A., Swanepoel, L. H. and Hunter, L. T. B. (2014). Failure of Research to Address the Rangewide Conservation Needs of Large Carnivores: Leopards in South Africa as a Case Study. pp. 3-11.

Bellemain, E., Swenson, J. E., Tallmon, D., Taberlet, P. and Brunberg, S. (2005). Estimating population size of elusive animals with DNA from hunter-collected feces: Four methods for brown bears. - Conservation Biology 19: 150-161.

Bischof, R., Swenson, J., Yoccoz, N., Mysterud, A. and Gimenez, O. (2009). The magnitude and selectivity of natural and multiple anthropogenic mortality causes in hunted brown bears. - Journal of Animal Ecology 78: 656-665.

Björnprojektet. (2015). Publications. - Skandinaviska Björnprojektet. http://bearproject.info/publications/.

Blanchard, B. M. and Knight, R. R. (1991). Movements of Yellowstone grizzly bears. - Biological Conservation 58: 41-67.

Blouin, M. S. (2003). DNA- based methods for pedigree reconstruction and kinship analysis in natural populations. - Trends in Ecology and Evolution 18: 503-511.

Brumfield, R. T., Beerli, P., Nickerson, D. A. and Edwards, S. V. (2003). The utility of single nucleotide polymorphisms in inferences of population history. - Trends in Ecology and Evolution 18: 249-256.

Calus, M. P. L., Mulder, H. A. and Bastiaansen, J. W. M. (2011). Identification of Mendelian inconsistencies between SNP and pedigree information of sibs. - Genetics, selection, evolution : GSE 43: 34.

Colwell, R. K. and Coddington, J. A. (1994). Estimating Terrestrial Biodiversity through Extrapolation. - Philosophical Transactions of the Royal Society B: Biological Sciences 345: 101-118.

Creel, S. and Rosenblatt, E. (2013). Using pedigree reconstruction to estimate population size: genotypes are more than individually unique marks. - Ecology and Evolution 3: 1294-1304.

Creel, S., Sands, J. L., Rotella, J., Spong, G., Zeigle, J., Joe, L., Murphy, K. M. and Smith, D. (2003). Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. - Molecular Ecology 12: 2003-2009.

Dahle, B. and Swenson, J. E. (2003). Home ranges in adult Scandinavian brown bears (Ursus arctos): Effect of mass, sex, reproductive category, population density and habitat type. - Journal of Zoology 260: 329-335.

Deyoung, R. W. and Honeycutt, R. L. (2005). THE MOLECULAR TOOLBOX: GENETIC TECHNIQUES IN WILDLIFE ECOLOGY AND MANAGEMENT. - Journal of Wildlife Management 69: 1362-1384.

Earl, D. and vonHoldt, B. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. - Conservation Genet Resour 4: 359-361.
Edwards, M. A. and Derocher, A. E. (2015). Mating-related behaviour of grizzly bears inhabiting marginal habitat at the periphery of their North American range. - Behavioural Processes 111: 75-83.
Eggert, L. S., Eggert, J. A. and Woodruff, D. S. (2003). Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. - Molecular Ecology 12: 1389-1402.
Ericsson, G. and Heberlein, T. A. (2003). Attitudes of hunters, locals, and the general public in Sweden now that the wolves are back. - Biological Conservation 111: 149-159.
ESRI. (2014). ArcGIS Desktop: Release 10. - Redlands, CA: Environmental Systems Research Institute.
Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure : a simulation study. - Molecular Ecology 14: 2611-2620.
Fahlen, J. (2014). SNP-based conservation genetics of the southern Swedish brown bear (Ursus arctos). Department of Wildlife, Fish, and Environmental Studies. - Sveriges lantbruksuniversitet (SLU).
Frantz, A. C., Roper, T. J., Schaul, M., Pope, L. C., Fack, F., Muller, C. P. and Schley, L. (2004). Estimating population size by genotyping remotely plucked hair: The Eurasian badger. - Journal of Applied Ecology 41: 985-995.
Frantzen, M. A. J., Silk, J. B., Ferguson, J. W. H., Wayne, R. K. and Kohn, M. H. (1998). Empirical evaluation of preservation methods for faecal DNA. - Molecular Ecology 7: 1423-1428.
Glenn, L. P. and Miller, L. H. (1980). Seasonal movements of an Alaska Peninsula brown bear population. - International Conference on Bear Research and Management 4: 307-312.
Guillot, G., Mortier, F. and Estoup, A. (2005). Geneland : a computer package for landscape genetics. - Molecular Ecology Notes 5: 712-715.
Guillot, G., Santos, F. and Estoup, A. (2015). Package 'Geneland'. Detection of structure from multilocus genetic data. - http://cran.r-project.org/web/packages/Geneland/Geneland.pdf
Hess, M. A., Rhydderch, J. G., Leclair, L. L., Buckley, R. M., Kawase, M. and Hauser, L. (2012). Estimation of genotyping error rate from repeat genotyping, unintentional recaptures and known parent–offspring comparisons in 16 microsatellite loci for brown rockfish (Sebastes auriculatus. - Molecular Ecology Resources 12: 1114-1123.
Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. - Biological Journal of the Linnean Society 68: 87-112.
Hirata, D., Mano, T., Abramov, A. V., Baryshnikov, G. F., Kosintsev, P. A., Vorobiev, A. A., Raichev, E. G., Tsunoda, H., Kaneko, Y., Murata, K., Fukui, D. and Masuda, R. (2013). Molecular Phylogeography of the Brown Bear (Ursus arctos) in Northeastern Asia Based on Analyses of Complete Mitochondrial DNA Sequences. - Molecular Biology and Evolution 30: 1644-1652.
Huber, D. and Roth, H. U. (1993). Movements of European brown bears in Croatia. - Acta Theriologica 38: 151-159.
Karanth, K. U. and Chellam, R. (2009). Carnivore conservation at the crossroads. Oryx, pp. 1-2.

Kellert, S. R., Black, M., Rush, C. R. and Bath, A. J. (1996). Human culture and large carnivore conservation in North America. - Conservation Biology 10: 977-990.
Kindberg, J. (2010). Monitoring and management of the Swedish brown bear (Ursus arctos) population.
Kindberg, J., Ericsson, G. and Swenson, J. (2009). Monitoring rare or elusive large mammals using effort-corrected voluntary observers. - Biological Conservation 142: 159-165.
Kindberg, J. and Swenson, J. E. (2013). Beräkning av björnstammens storlek i Värmland, Dalarnas och Gävleborgs län. - Scandinavian Brown Bear Research Project, report 2013:4.
Kindberg, J., Swenson, J. E., Ericsson, G., Bellemain, E., Miquel, C. and Taberlet, P. (2011). Estimating population size and trends of the Swedish brown bear Ursus arctos population. - Wildlife Biology 17: 114-123.
Kohn, M. H. and Wayne, R. K. (1997). Facts from feces revisited. - Trends in Ecology & Evolution 12: 223-227.
Kohn, M. H., Wayne, R. K., York, E. C., Kamradt, D. A., Haught, G. and Sauvajot, R. M. (1999). Estimating population size by genotyping faeces. - Proceedings of the Royal Society of London - B. Biological Sciences 266: 657-663.
Korsten, M., Ho, S., Davison, J., PÄhn, B., Vulla, E., Roht, M., Tumanov, I., Kojola, I., Andersone-Lilley, Z., Ozolins, J., Pilot, M., Mertzanis, Y., Giannakopoulos, A., Vorobiev, A., Markov, N., Saveljev, A., Lyapunova, E., Abramov, A., MÄnnil, P., Valdmann, H., Pazetnov, S., Pazetnov, V., RÕkov, A. and Saarma, U. (2009). Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: A general demographic model for mammals? - Molecular Ecology 18: 1963-1979.
Krebs, C. J. (1999). Ecological methodology. - Menlo Park, Calif. : Benjamin/Cummings.
Krofel, M., Filacorda, S. and Jerina, K. (2010). Mating-related movements of male brown bears on the periphery of an expanding population. - Ursus 21: 23-29.
Liberg, O., Andren, H., Pedersen, H. C., Sand, H., Sejberg, D., Wabakken, P., Akesson, M. and Bensch, S. (2005). Severe inbreeding depression in a wild wolf (Canis lupus) population. - Biology Letters 1: 17-20.
Logan, K. A. (2001). Desert puma evolutionary ecology and conservation of an enduring carnivore. - In: Sweanor, L. L. (ed.). - Washington, DC : Island Press, p. 119.
Lynch, M. and Ritland, K. (1999). Estimation of pairwise relatedness with molecular markers. - Genetics 152: 1753.
Manel, S., Bellemain, E., Swenson, J. E. and Francois, O. (2004). Assumed and inferred spatial structure of populations: The Scandinavian brown bears revisited. - Molecular Ecology 13: 1327-1331.
Manel, S., Holderegger, R. and Manel, S. (2013). Ten years of landscape genetics. - Trends in Ecology & Evolution 28: 614.
Meirmans, P. G. (2015). Seven common mistakes in population genetics and how to avoid them. - Molecular Ecology DOI: 10.1111/mec.13243.
Mesnick, S. L., Taylor, B. L., Archer, F. I., Martien, K. K., Hancock-Hanser, B. L., Pease, V. L., Robertson, K. M., Morin, P. A., Treviño, S. E., Moreno Medina, S. C., Straley, J. M., Baird, R. W., Calambokidis, J., Schorr, G. S., Wade, P., Burkanov, V., Lunsford, C. R. and Rendell, L. (2011). Sperm whale population structure in the eastern and central North Pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA. - Molecular Ecology Resources 11: 278-298.

Mills, M. G. L., Juritz, J. M. and Zucchini, W. (2001). Estimating the size of spotted hyaena (Crocuta crocuta) populations through playback recordings allowing for non-response. - Animal Conservation 4: 335-343.
Morin, P. A., Luikart, G., Wayne, R. K. and the Snp workshop group, R. K. (2004). SNPs in ecology, evolution and conservation. - Trends in Ecology & Evolution 19: 208-216.
Morin, P. A., Martien, K. K. and Taylor, B. L. (2009). Assessing statistical power of SNPs for population structure and conservation studies. - Molecular Ecology Resources 9: 66-73.
Morin, P. A. and McCarthy, M. (2007). Highly accurate SNP genotyping from historical and low-quality samples. - Mol. Ecol. Notes 7: 937-946.
Morner, T., Eriksson, H., Brojer, C., Nilsson, K., Uhlhorn, H., Agren, E., Segerstad, C. H., Jansson, D. S. and Gavier-Widen, D. (2005). Diseases and mortality in free-ranging brown bear (Ursus arctos), gray wolf (Canis lupus), and Wolverine (Gulo gulo) in Sweden. - Journal of Wildlife Diseases 41: 298-303.
Nilsson, T. (2013). Population viability analyses of the Scandinavian populations of bear (Ursus arctos) lynx (Lynx lynx) and wolverine (Gulo gulo). Report 6549. - Swedish Environmental Protection Agency.
Norman, A. J., Street, N. R. and Spong, G. (2013). De Novo SNP Discovery in the Scandinavian Brown Bear (Ursus arctos). - PLoS One 8.
Petit, E. and Valiere, N. (2006). Estimating Population Size with Noninvasive Capture-Mark-Recapture Data. - Conservation Biology 20: 1062-1073.
Pritchard, J. K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. - Genetics 155: 945.
Pritchard, J. K., Wen, X. and Falush, D. (2010). Documentation for structure software: version 2.3. - University of Chicago.
R Development Core Team. (2008). R: A language and environment for statistical computing. - R Foundation for Statistical Computing.
Regeringskansliet. (2014). Sweden's Environmental Quality Objectives. - Swedish Government Offices. Stockholm. http://www.government.se/sb/d/5775
Riester, M., Stadler, P. F. and Klemm, K. (2009). FRANz: reconstruction of wild multi-generation pedigrees. - Bioinformatics 25: 2134-2139.
Rieux, A., Halkett, F., De Lapeyre de Bellaire, L., Zapater, M. F., Rousset, F., Ravigné, V. and Carlier, J. (2011). Inferences on pathogenic fungus population structures from microsatellite data: new insights from spatial genetics approaches. - Inferences on pathogenic fungus population structures from microsatellite data: new insights from spatial genetics approaches 20: 1661-1674.
Ross, P. I. and Jalkotzy, M. G. (1992). Characteristics of a hunted population of cougars in southwestern Alberta. - Journal of Wildlife Management 56: 417-426.
Rousset, F. o. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. - genepop'007: a complete re-implementation of the genepop software for Windows and Linux 8: 103-106.
Saarma, U., Ho, S. Y. W., Pybus, O. G., Kaljuste, M., Tumanov, I. L., Kojola, I., Vorobiev, A. A., Markov, N. I., Saveljev, A. P., Valdmann, H., Lyapunova, E. A., Abramov, A. V., Mannil, P., Korsten, M., Vulla, E., Pazetnov, S. V., Pazetnov, V. S., Putchkovskiy, S. V. and Rokov, A. M. (2007). Mitogenetic structure of brown bears (Ursus arctos L.) in northeastern Europe and a new time frame for the formation of European brown bear lineages. - Molecular Ecology 16: 401-413.
Saarma, U. and Kojola, I. (2007). Matrilineal genetic structure of the brown bear population in Finland. - Ursus 18: 30-37.

Saether, B. E., Engen, S., Swenson, J. E., Bakke, O. and Sandegren, F. (1998). Assessing the viability of Scandinavian brown bear, Ursus arctos, populations: the effects of uncertain parameter estimates. - Assessing the viability of Scandinavian brown bear, Ursus arctos, populations: the effects of uncertain parameter estimates 83: 403-416.

SAS Institute, I. JMP Pro version 11.0.0.

Schregel, J., Kopatz, A., Hagen, S. B., Broseth, H., Smith, M. E., Wikan, S., Wartiainen, I., Aspholm, P. E., Aspi, J., Swenson, J. E., Makarova, O., Polikarpova, N., Schneider, M., Knappskog, P. M., Ruokonen, M., Kojola, I., Tirronen, K. F., Danilov, P. I. and Eiken, H. G. (2012). Limited gene flow among brown bear populations in far Northern Europe? Genetic analysis of the east-west border population in the Pasvik Valley. - Molecular Ecology 21: 3474-3488.

Schwartz, M. K., Luikart, G. and Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. - Trends in Ecology & Evolution 22: 25-33.

Sripichai, O. and Fucharoen, S. (2007). Genetic polymorphisms and implications for human diseases. - Journal of the Medical Association of Thailand = Chotmaihet thangphaet 90: 394.

Steyaert, S. M. J. G., Endrestøl, A., Hackländer, K., Swenson, J. E. and Zedrosser, A. (2012). The mating system of the brown bear Ursus arctos. - Mammal Review 42: 12-34.

Støen, O.-G., Bellemain, E., Sæbø, S. and Swenson, J. (2005). Kin-related spatial structure in brown bears Ursus arctos. - Behav Ecol Sociobiol 59: 191-197.

Støen, O.-G., Zedrosser, A., Sæbø, S. and Swenson, J. (2006). Inversely density-dependent natal dispersal in brown bears Ursus arctos. - Oecologia 148: 356-364.

Sutherland, W. (2006). Ecological census techniques : a handbook. - Cambridge : Cambridge University Press.

Swenson, J. E., Sandegren, F., Bjarvall, A., Soderberg, A., Wabakken, P. and Franzen, R. (1994). Size, trend, distribution and conservation of the brown bear Ursus arctos population in Sweden. - Biological Conservation 70: 9-17.

Swenson, J. E., Sandegren, F., Brunberg, S. and Segerstrom, P. (2001). Factors associated with loss of brown bear cubs in Sweden. - Ursus 12: 69-80.

Swenson, J. E., Taberlet, P. and Bellemain, E. (2011). Genetics and conservation of European brown bears Ursus arctos. - Mammal Review 41: 87-98.

Taberlet, P. and Bouvet, J. (1994). Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear Ursus arctos in Europe. - Proceedings of the Royal Society of London - B. Biological Sciences 255: 195-200.

Taberlet, P., Luikart, G. and Waits, L. P. (1999). Noninvasive genetic sampling: Look before you leap. - Trends in Ecology and Evolution 14: 323-327.

Taberlet, P., Swenson, J. E., Sandegren, F. and Bjarvall, A. (1995). Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear Ursus arctos in Scandinavia. - Conservation Biology 9: 1255-1261.

Valière, N. (2002). gimlet : a computer program for analysing genetic individual identification data. - Molecular Ecology Notes 2: 377-379.

Vié, J.-C., Hilton-Taylor, C. and Stuart, S. N. (2009). Wildlife in a changing world ; IUCN red list of threatened species. 2008: an analysis of the 2008 IUCN red list of threatened species. Analysis of the 2008 IUCN red list of threatened species. - Gland, Switzerland ; Barcelona, Spain: IUCN: Lynx Edicions.

Vignal, A., Milan, D., SanCristobal, M. and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. - Genetics, Selection, Evolution : GSE 34: 275-305.

Waits, L., Taberlet, P., Swenson, J. E., Sandegren, F. and Franzen, R. (2000). Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (Ursus arctos). - Molecular Ecology 9: 421-431.
Waits, L. P., Luikart, G. and Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. - Molecular Ecology 10: 249-256.
Waits, L. P. and Paetkau, D. (2005). Noninvasive genetic sampling tools for wildlife biologists: A review of applications and recommendations for accurate data collection. - Journal of Wildlife Management 69: 1419-1433.
Wang, J. and Santure, A. W. (2009). Parentage and sibship inference from multilocus genotype data under polygamy. - Genetics 181: 1579.
Waples, R. S. (2015). Testing for Hardy-Weinberg Proportions: Have We Lost the Plot? - Journal of Heredity 106: 1-19.
Weir, B. S. and Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. - Evolution 38: 1358-1370.
White, G. and Burnham, K. (1999). Program MARK: survival estimation from populations of marked animals. - Bird Study 46: 120-139.
Wilson, G. J. and Delahay, R. J. (2001). A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. - Wildlife Research 28: 151-164.
Zedrosser, A., Steyaert, S. M. J. G., Gossow, H. and Swenson, J. E. (2011). Brown bear conservation and the ghost of persecution past. - Biological Conservation 144: 2163-2170.
Zimmermann, A., Baker, N., Inskip, C., Linell, J., Marchini, S., Odden, J., Rasmussen, G. and Treves, A. (2010). Contemporary Views of Human–Carnivore Conflicts on Wild Rangelands. - In: Toit, J. T. d., et al. (eds.), Wild Rangelands: Conserving Wildlife While Maintaining Livestock in Semi-Arid Ecosystems. Blackwell Publishing, pp. 129-151.
Zuercher, G. L., Gipson, P. S. and Stewart, G. C. (2003). Identification of Carnivore Feces by Local Peoples and Molecular Analyses. - Wildlife Society Bulletin 31: 961-970.

9 Appendices

Appendix I Dalarna-Gävleborg Pedigree

ID	SIRE	DAM
W12001	W12075	*
W12002	*	*
W12003	*	W12117
W12004	*	*
W12005	W12075	W12008
W12006	*	*
W12007	*	*
W12008	*	W12004
W12009	*	W12008
W12010	W12106	*
W12011	*	*
W12012	W12047	W12050
W12013	W12032	W12038
W12014	*	*
W12015	X12170	*
W12016	*	*
W12017	*	*
W12018	*	W12052
W12019	X12065	*
W12020	W12176	W12021
W12021	X12023	*
W12023	*	*
W12024	*	*
W12025	*	W12185
W12026	*	W12182
W12027	*	*
W12028	*	W12124
W12029a	*	*
W12030	*	*
W12032	*	*
W12033	*	*
W12034	W12023	W12061
W12035	*	*
W12036	*	*
W12037	W12032	W12038
W12038	*	W12117
W12039	*	*
W12040	W12044	W12039
W12041	W12044	W12039
W12042	*	*
W12043	W12106	W12052
W12044	*	*
W12045	*	W12048
W12046	*	W12025
W12047	W12086	X12035
W12048	W12176	W12108
W12049	*	*
W12050	*	W12202
W12051	*	W12048
W12052	W12144	*
W12053	*	*
W12054	X12131	*
W12055	*	*
W12056	*	*
W12057	*	W12038
W12058	*	*
W12059	*	*
W12060	W12047	W12019
W12061	*	W12121
W12062	W12032	W12038

ID	SIRE	DAM
W12063	*	W12064
W12064	*	*
W12065	*	W12052
W12066	*	*
W12067	*	*
W12068	*	*
W12069	*	X12145
W12070	*	*
W12071	W12111	*
W12072	W12178	*
W12073	W12011	*
W12074	*	W12082
W12075	*	*
W12076	*	*
W12077	W12073	W12001
W12078	*	*
W12079	W12075	W12084
W12080	W12033	*
W12081	W12075	W12084
W12082	W12078	*
W12083	W12075	W12080
W12084	*	*
W12085	W12073	W12001
W12086	*	*
W12087	*	X12195
W12088	*	*
W12089	*	W12165
W12090	W12104	*
W12091	X12045	W12120
W12092	*	W12038
W12093	*	W12002
W12094	*	*
W12095	*	*
W12096	*	W12182
W12097	W12113	*
W12098	*	*
W12099	X12114	*
W12100	X12045	W12120
W12101	W12075	*
W12102	*	*
W12103	*	*
W12104	*	*
W12105	X12114	W12112
W12106	W12171	*
W12107	W12072	W12029a
W12108	*	*
W12109	*	*
W12110	*	*
W12111	*	*
W12112	*	W12170
W12113	*	*
W12114	W12154	*
W12115	W12047	W12019
W12116	*	W12117
W12117	*	W12007
W12118	W12097	W12061
W12119	*	W12002
W12120	*	*
W12121	*	W12007
W12122	W12176	W12021

ID	SIRE	DAM
W12123	W12097	W12061
W12124	*	W12196
W12125	*	*
W12126	*	*
W12127	X12104	*
W12128	W12023	W12151
W12129	*	X12062
W12130	*	*
W12131	*	*
W12132	*	*
W12133	*	*
W12134	*	*
W12135	*	W12197
W12136	X12180	*
W12137	*	*
W12138	*	*
W12139	*	W12025
W12140	W12104	W12028
W12141	W12075	W12008
W12142	*	*
W12143	*	*
W12144	*	*
W12145	*	W12135
W12146	*	*
W12147	X12131	*
W12148	*	*
W12149	*	W12087
W12150	*	*
W12151	*	W12120
W12152	*	W12038
W12153	W12160	*
W12154	*	*
W12155	*	*
W12156	*	W12182
W12157	W12176	W12108
W12158	*	*
W12159	*	*
W12160	*	*
W12161	*	*
W12162	*	*
W12163	*	*
W12164	*	*
W12165	*	*
W12166	W12047	W12131
W12167	*	*
W12168	*	*
W12169	*	*
W12170	*	*
W12171	*	*
W12172	W12106	W12052
W12173	*	*
W12174	X12158	*
W12175	*	*
W12176	*	*
W12177	W12072	W12029a
W12178	*	*
W12179	*	W12052
W12180	X12104	W12021
W12181	*	W12186
W12182	W12024	*

ID	SIRE	DAM
W12183	*	W12153
W12184	W12178	W12108
W12185	*	X12145
W12186	*	W12198
W12188	*	W12052
W12189	*	W12019
W12190	X12125	*
W12191	*	*
W12192	*	*
W12193	*	*
W12194	*	*
W12195	*	*
W12196	W12068	*
W12197	*	W12019
W12198	*	W12090
W12199	*	W12025
W12200	W12176	*
W12201	*	X12070
W12202	*	*
W12203	W12154	W12211
W12204	*	*
W12205	*	*
W12207	X12192	*
W12208	*	X12187
W12209	*	*
W12210	*	*
W12211	*	W12010
W12212	W12106	W12029a
W12213	W12178	W12108
W12214	*	*
W12215	X12033	*
W12216	*	W12090
W12218	*	*
W12219	*	*
W12220	*	*
W12221	*	W12165
W12001	*	*
X12002	X12186	*
X12003	*	*
X12004	X12112	X12169
X12005	*	*
X12006	*	*
X12007	*	*
X12008	*	*
X12009	X12010	*
X12010	*	*
X12011	*	*
X12012	*	X12171
X12013	X12191	*
X12014	*	*
X12015	X12133	X12154
X12016	X12036	*
X12017	*	X12142
X12018	*	*
X12019	X12192	*
X12020	*	*
X12021	X12170	X12128
X12022	*	*
X12023	*	*
X12024	*	X12175

ID	SIRE	DAM
X12025	*	W12135
X12026	X12025	X12165
X12027	*	X12194
X12028	*	*
X12029	*	*
X12030	X12104	*
X12031	*	*
X12032	*	*
X12033	*	*
X12034	W12219	*
X12035	W12094	*
X12036	X12206	*
X12037	*	*
X12038	*	*
X12039	*	*
X12040	X12058	X12195
X12041	*	X12086
X12042	*	*
X12043	X12126	*
X12044	*	X12066
X12045	*	*
X12046	X12127	*
X12047	X12025	*
X12048	W12035	*
X12049	*	X12211
X12050	*	*
X12051	*	*
X12052	*	*
X12053	W12219	*
X12054	*	X12057
X12055	X12131	X12110
X12056	X12125	X12006
X12057	*	X12066
X12058	*	*
X12059	*	*
X12060	*	*
X12061	*	*
X12062	*	W12185
X12063	*	X12094
X12064	*	*
X12065	*	*
X12066	X12203	*
X12067	*	*
X12068	X12036	X12209
X12069	*	X12183
X12070	*	X12013
X12071	*	X12200
X12072	X12010	*
X12073	*	*
X12074	*	X12153
X12075	*	*
X12076	*	X12034
X12077	*	X12022
X12078	*	*
X12079	*	X12183
X12080	*	*
X12081	*	X12171
X12082	*	*
X12083	*	W12025
X12084	*	*

ID	SIRE	DAM
X12085	*	*
X12086	X12188	*
X12087	*	*
X12088	X12185	*
X12089	X12172	X12154
X12090	*	*
X12091	*	X12187
X12092	*	*
X12093	*	X12144
X12094	*	X12093
X12095	*	*
X12096	X12158	X12093
X12097	*	X12120
X12098	*	*
X12099	X12158	X12093
X12100	*	W12136
X12101	*	*
X12102	*	*
X12103	X12078	X12150
X12104	X12098	*
X12105	X12137	*
X12106	X12011	*
X12107	*	*
X12108	*	*
X12109	X12032	*
X12110	*	*
X12111	W12035	*
X12112	*	*
X12113	X12011	X12035
X12114	*	*
X12115	*	*
X12116	*	*
X12117	W12011	X12194
X12118	*	*
X12119	*	X12120
X12120	*	X12146
X12121	*	*
X12122	*	*
X12123	*	*
X12124	X12088	*
X12125	*	*
X12126	*	*
X12127	X12173	*
X12128	W12174	*
X12129	X12125	X12156
X12130	X12170	X12128
X12131	*	*
X12132	*	X12092
X12133	*	*
X12134	X12082	*
X12135	*	*
X12136	*	*
X12137	*	*
X12138	*	*
X12139	X12168	*
X12140	*	*
X12141	X12172	X12154
X12142	*	*
X12143	*	X12076
X12144	X12131	*

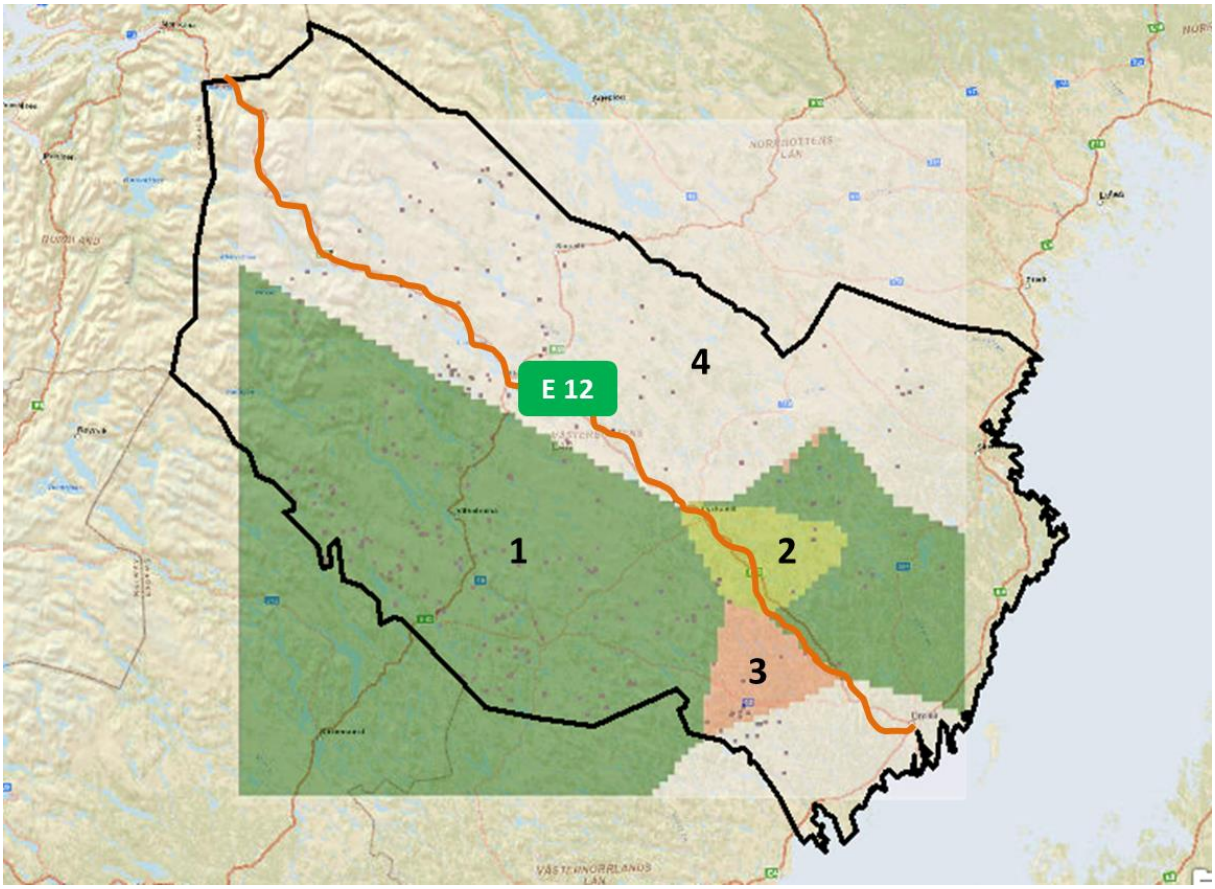
ID	SIRE	DAM
X12145	W12194	*
X12146	*	X12193
X12147	*	*
X12148	*	X12198
X12149	*	*
X12150	*	*
X12151	X12052	X12183
X12152	*	X12014
X12153	X12036	X12209
X12154	*	*
X12155	*	*
X12156	*	*
X12157	*	X12134
X12158	X12131	*
X12159	*	X12128
X12160	*	X12062
X12161	*	*
X12162	*	*
X12163	*	*
X12164	X12033	*
X12165	W12194	*
X12166	X12168	*
X12167	X12025	*
X12168	*	X12002
X12169	*	X12128
X12170	*	*
X12171	X12216	*
X12172	*	*
X12173	*	*
X12174	*	X12187
X12175	X12131	*
X12176	*	*
X12177	X12025	X12076
X12178	X12025	*
X12179	X12127	*
X12180	*	*
X12181	X12078	X12165
X12182	*	*
X12183	*	X12134
X12184	X12137	X12071
X12185	*	*
X12186	X12104	W12136
X12187	*	X12076
X12188	*	*
X12189	*	*
X12190	X12058	X12195
X12191	*	*
X12192	X12218	*
X12193	X12199	*
X12194	X12135	*
X12195	*	X12128
X12196	*	*
X12197	X12112	X12169
X12198	*	X12076
X12199	*	*
X12200	*	W12039
X12201	*	*
X12202	*	*
X12203	*	*
X12204	*	X12174

ID	SIRE	DAM
X12205	*	*
X12206	*	*
X12207	X12052	X12183
X12208	W12095	*
X12209	*	X12204
X12210	*	*
X12211	*	*
X12212	*	W12185
X12213	*	*
X12214	*	X12202
X12215	*	X12202
X12216	*	*
X12217	X12011	X12035
X12218	*	*

Appendix II Västerbotten Pedigree

ID	SIRE	DAM
AC2014-001	AC2014-148	AC2014-223
AC2014-003	*	AC2014-019
AC2014-004	*	*
AC2014-005	AC2014-017	*
AC2014-006	*	*
AC2014-007	*	*
AC2014-008	*	*
AC2014-009	AC2014-161	*
AC2014-010	*	AC2014-186
AC2014-011	AC2014-010	AC2014-102
AC2014-012	*	*
AC2014-013	*	*
AC2014-014	AC2014-149	*
AC2014-015	*	AC2014-132
AC2014-016	*	*
AC2014-017	*	*
AC2014-018	AC2014-081	AC2014-125
AC2014-019	AC2014-135	*
AC2014-020	*	AC2014-115
AC2014-021	*	*
AC2014-022	*	*
AC2014-023	*	*
AC2014-024	*	AC2014-115
AC2014-025	AC2014-022	*
AC2014-026	*	AC2014-015
AC2014-027	*	AC2014-160
AC2014-028	*	*
AC2014-029	*	*
AC2014-030	AC2014-240	*
AC2014-031	*	AC2014-005
AC2014-032	AC2014-107	AC2014-159
AC2014-033	*	*
AC2014-034	AC2014-100	*
AC2014-035	AC2014-161	AC2014-171
AC2014-036	*	*
AC2014-037	*	AC2014-106
AC2014-038	*	*
AC2014-039	*	*
AC2014-040	AC2014-170	*
AC2014-041	*	*
AC2014-042	*	AC2014-123
AC2014-043	AC2014-142	*
AC2014-044	*	*
AC2014-045	AC2014-065	AC2014-111
AC2014-046	*	*
AC2014-047	*	*
AC2014-048	*	*
AC2014-049	AC2014-045	AC2014-102
AC2014-050	*	AC2014-057
AC2014-051	AC2014-052	*
AC2014-052	*	*
AC2014-053	AC2014-043	AC2014-212
AC2014-054	*	*
AC2014-055	*	AC2014-177
AC2014-056	*	AC2014-171
AC2014-057	*	AC2014-163
AC2014-058	AC2014-081	*
AC2014-059	*	*
AC2014-060	AC2014-230	*
AC2014-061	*	AC2014-212
AC2014-062	*	*
AC2014-063	*	AC2014-019
AC2014-064	*	*
AC2014-065	*	*
AC2014-066	*	*
AC2014-067	*	*
AC2014-068	AC2014-148	AC2014-223
AC2014-069	*	*
AC2014-070	*	*
AC2014-071	*	*
AC2014-072	*	*
AC2014-073	*	*
AC2014-074	AC2014-146	*
AC2014-075	*	AC2014-077
AC2014-076	AC2014-164	*
AC2014-077	*	*
AC2014-078	AC2014-154	AC2014-016
AC2014-079	*	*
AC2014-080	AC2014-048	AC2014-096
AC2014-081	*	*
AC2014-082	*	*
AC2014-083	*	AC2014-096
AC2014-084	*	*
AC2014-085	AC2014-013	*
AC2014-086	*	*
AC2014-087	*	*
AC2014-088	AC2014-052	*
AC2014-089	*	*
AC2014-090	AC2014-107	*
AC2014-091	*	AC2014-088
AC2014-092	*	AC2014-101
AC2014-093	*	AC2014-171
AC2014-094	*	*
AC2014-095	AC2014-107	AC2014-092
AC2014-096	AC2014-087	*
AC2014-097	*	*
AC2014-098	*	AC2014-246
AC2014-099	*	AC2014-166
AC2014-100	AC2014-031	*
AC2014-101	*	*
AC2014-102	*	*
AC2014-103	AC2014-031	AC2014-137
AC2014-104	*	AC2014-233
AC2014-105	*	*
AC2014-106	AC2014-122	*
AC2014-107	*	*
AC2014-108	*	*
AC2014-109	*	*
AC2014-110	*	*
AC2014-111	*	*
AC2014-112	AC2014-070	*
AC2014-113	AC2014-104	AC2014-115
AC2014-114	*	AC2014-090
AC2014-115	AC2014-259	*
AC2014-116	*	*
AC2014-117	AC2014-010	AC2014-102
AC2014-118	*	*
AC2014-119	AC2014-107	AC2014-114
AC2014-120	AC2014-006	*
AC2014-121	AC2014-098	*
AC2014-122	*	*
AC2014-123	AC2014-162	*
AC2014-124	*	*
AC2014-125	*	*
AC2014-126	AC2014-081	AC2014-125
AC2014-128	*	*
AC2014-129	*	*
AC2014-130	*	*
AC2014-131	AC2014-221	*
AC2014-132	AC2014-063	*
AC2014-133	*	AC2014-236
AC2014-134	*	*
AC2014-135	*	*
AC2014-136	*	*
AC2014-137	*	AC2014-030
AC2014-138	AC2014-054	AC2014-060
AC2014-139	*	AC2014-192
AC2014-140	AC2014-154	AC2014-016
AC2014-141	*	*
AC2014-142	*	AC2014-163
AC2014-143	*	*
AC2014-144	*	AC2014-077
AC2014-145	AC2014-052	*
AC2014-146	*	*
AC2014-147	AC2014-079	*
AC2014-148	*	*
AC2014-149	*	*
AC2014-150	*	*
AC2014-151	AC2014-231	*
AC2014-152	*	*
AC2014-153	*	AC2014-015
AC2014-154	*	*
AC2014-155	*	*
AC2014-156	*	*
AC2014-157	AC2014-227	*
AC2014-158	*	AC2014-153
AC2014-159	*	*
AC2014-160	*	*
AC2014-161	*	*
AC2014-162	AC2014-178	*
AC2014-163	AC2014-059	*
AC2014-164	*	*
AC2014-165	AC2014-189	*
AC2014-166	*	AC2014-084
AC2014-167	*	AC2014-212
AC2014-168	AC2014-054	AC2014-210
AC2014-169	*	AC2014-249
AC2014-170	*	*
AC2014-171	AC2014-157	*
AC2014-172	*	*
AC2014-173	*	AC2014-124
AC2014-174	*	AC2014-003
AC2014-175	*	AC2014-261
AC2014-176	AC2014-142	AC2014-186
AC2014-177	AC2014-142	AC2014-186
AC2014-178	AC2014-054	AC2014-210
AC2014-179	*	AC2014-005
AC2014-180	AC2014-031	AC2014-137
AC2014-181	*	AC2014-159
AC2014-182	*	AC2014-168
AC2014-183	AC2014-048	AC2014-096
AC2014-184	*	*
AC2014-185	*	AC2014-096
AC2014-186	AC2014-164	*
AC2014-187	*	*
AC2014-188	*	*
AC2014-189	*	*
AC2014-190	AC2014-131	*
AC2014-191	AC2014-129	*
AC2014-192	AC2014-108	*
AC2014-193	*	AC2014-209
AC2014-194	AC2014-063	AC2014-027
AC2014-195	*	AC2014-166
AC2014-196	AC2014-013	*
AC2014-197	*	*
AC2014-198	*	AC2014-056
AC2014-199	AC2014-107	*
AC2014-200	AC2014-081	*
AC2014-201	*	AC2014-263
AC2014-202	AC2014-148	AC2014-074
AC2014-203	AC2014-148	AC2014-074
AC2014-204	*	AC2014-121
AC2014-205	AC2014-251	*
AC2014-206	*	AC2014-121
AC2014-207	*	*
AC2014-208	*	*
AC2014-209	AC2014-196	*
AC2014-210	*	*
AC2014-211	*	*
AC2014-212	*	*
AC2014-213	*	*
AC2014-214	*	AC2014-150
AC2014-215	*	AC2014-125
AC2014-216	*	*
AC2014-217	*	*
AC2014-218	AC2014-043	AC2014-212
AC2014-219	AC2014-107	*
AC2014-221	*	*
AC2014-222	*	*
AC2014-223	*	*
AC2014-224	*	*
AC2014-225	*	AC2014-125
AC2014-226	AC2014-152	*
AC2014-227	AC2014-081	*
AC2014-228	*	AC2014-132
AC2014-229	AC2014-081	AC2014-125
AC2014-230	*	*
AC2014-231	AC2014-107	AC2014-024
AC2014-232	AC2014-104	AC2014-233
AC2014-233	*	AC2014-177
AC2014-234	*	AC2014-075
AC2014-236	*	AC2014-125
AC2014-239	*	AC2014-050
AC2014-240	*	*
AC2014-242	*	*
AC2014-243	*	*
AC2014-244	*	AC2014-036
AC2014-245	*	AC2014-179
AC2014-246	*	AC2014-137
AC2014-247	*	*
AC2014-248	*	*
AC2014-249	*	AC2014-209
AC2014-250	*	*
AC2014-251	*	*
AC2014-252	AC2014-170	*
AC2014-253	*	AC2014-232
AC2014-254	*	AC2014-123
AC2014-255	*	AC2014-123
AC2014-256	*	*
AC2014-257	*	AC2014-025
AC2014-258	AC2014-140	*
AC2014-259	*	*
AC2014-260	*	*
AC2014-261	*	*
AC2014-262	AC2014-184	AC2014-271
AC2014-263	*	AC2014-034
AC2014-264	AC2014-148	AC2014-074
AC2014-265	AC2014-184	AC2014-271
AC2014-266	*	*
AC2014-267	AC2014-130	*
AC2014-268	*	AC2014-263
AC2014-269	AC2014-148	AC2014-223
AC2014-270	AC2014-081	*
AC2014-271	AC2014-063	AC2014-015
AC2014-272	*	AC2014-075

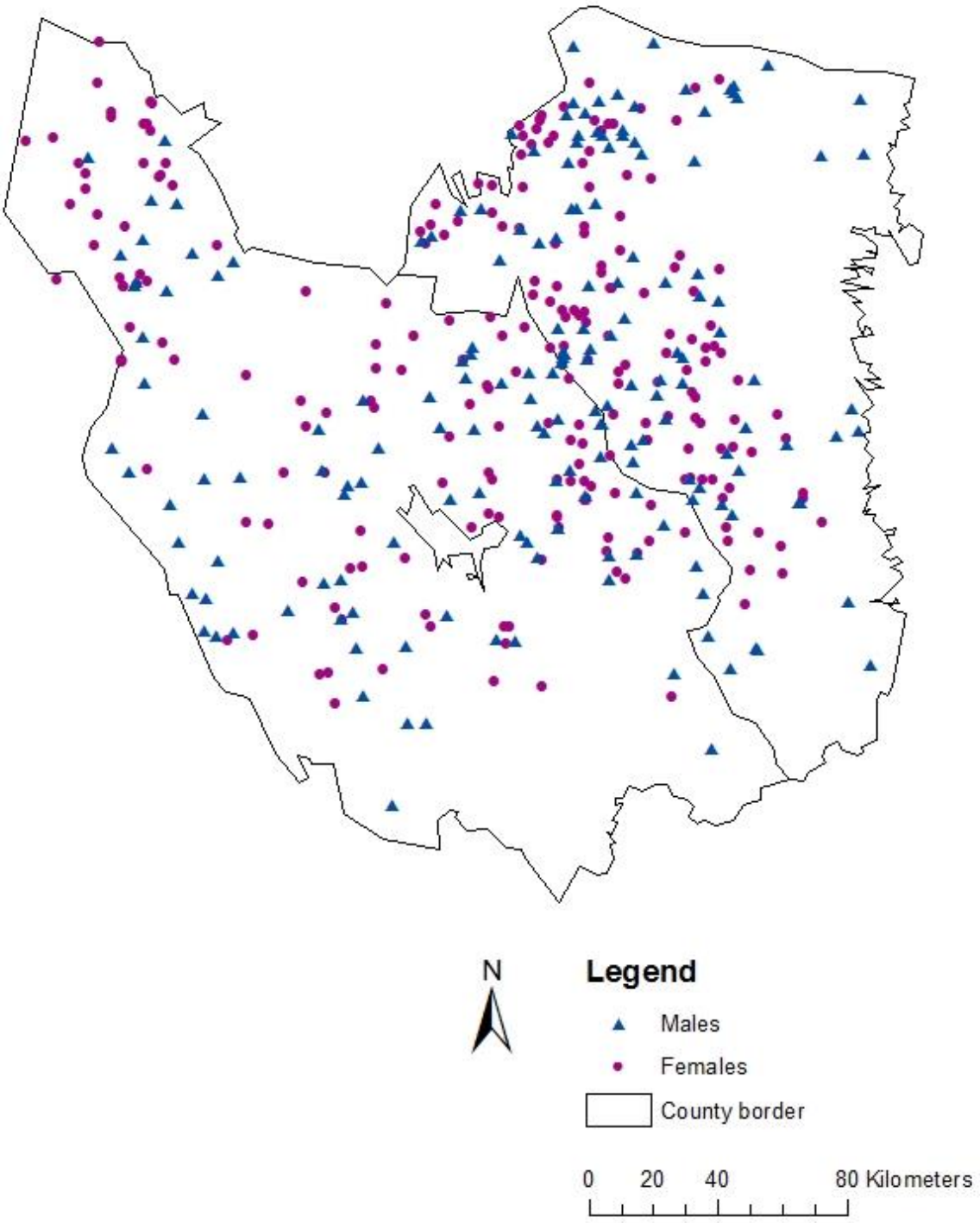
Appendix III Spatio-genetic population clusters and landscape features in Västerbotten



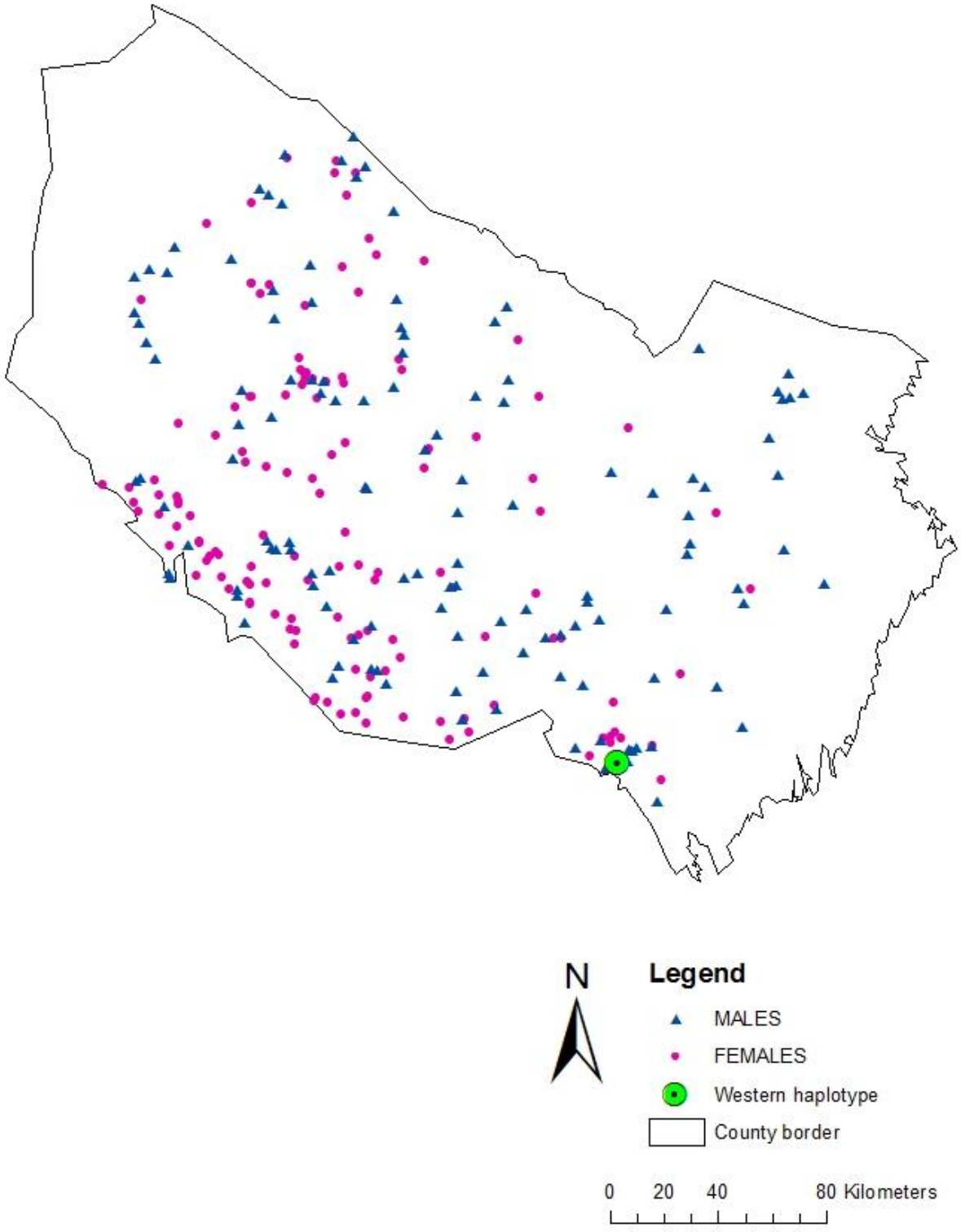
The photograph shows Highway E12 next to the Ume River. It should be noted that while the E12 is a major road in Västerbotten, it is usually unfenced and has only two lanes.

Appendix IV Locations (median centers) of male and female bears

Dalarna-Gävleborg



Västerbotten



SENASTE UTGIVNA NUMMER

2014:12 SNP-based conservation genetics of the southern Swedish brown bear (Ursus arctos) population
Författare: Joanna Fahlén

2014:13 Comparison of tree cavity abundance and characteristics in managed and unmanaged Swedish boreal forest.
Författare: Sophie Michon

2014:14 Habitat modeling for rustic bunting (Emberiza rustica) territories in boreal Sweden
Författare: Emil Larsson

2014:15 The Secret Role of Elephants - Mediators of habitat scale and within-habitat scale predation risk
Författare: Urza Flezar

2014:16 Movement ecology of Golden eagles (Aquila crysaetos) and risks associated with wind farm development
Författare: Rebecka Hedfors

2015:1 GIS-based modelling to predict potential habitats for black stork (Ciconia nigra) in Sweden
Författare: Malin Sörhammar

2015:2 The repulsive shrub – Impact of an invasive shrub on habitat selection by African large herbivores
Författare: David Rozen-Rechels

2015:3 Suitability analysis of a reintroduction of the great bustard (Otis tarda) to Sweden
Författare: Karl Fritzson

2015:4 AHA in northern Sweden – A case study
Conservation values of deciduous trees based on saproxylic insects
Författare: Marja Fors

2015:5 Local stakeholders' willingness to conduct actions enhancing a local population of Grey Partridge on Gotland – an exploratory interview study
Författare: Petra Walander

2015:6 Synchronizing migration with birth: An exploration of migratory tactics in female moose
Författare: Linnéa Näsén

2015:7 The impact of abiotic factors on daily spawning migration of Atlantic salmon (Salmo salar) in two north Swedish rivers
Författare: Anton Holmsten

2015:8 Restoration of white-backed woodpecker *Dendrocopos leucotos* habitats in central Sweden – Modelling future habitat suitability and biodiversity indicators
Författare: Niklas Trogen

Hela förteckningen på utgivna nummer hittar du på www.slu.se/viltfiskmiljo