European Journal of Wildlife Research (2025) 71:100
https://doi.org/10.1007/s10344-025-01982-9

RESEARCH

Estimating the population size of a semi-isolated moose (*Alces alces*) population from two sources of non-invasively collected DNA

Julia L. Jansson[1] · **Barbara Giles**[2] · **Göran Spong**[1]

Received: 28 March 2025 / Revised: 18 July 2025 / Accepted: 11 August 2025

Abstract

While non-invasive genetic methods have become increasingly important for estimating the abundance of wildlife populations, finding sufficient high-quality samples for accurate genotyping and population estimation remains a challenge. We tested whether salivary DNA from twigs browsed by moose (*Alces alces*) could complement fecal samples for individual identification and population size estimation using genetic mark-recapture. Browsed twigs and fecal samples were collected from two adjacent plateau mountains in Southern Sweden with a potentially isolated moose population. Twig samples were first genotyped with SNP (single nucleotide polymorphism) assays developed for cervid identification. The moose-positive twig and fecal samples were then genotyped on a SNP assay developed for individual identification. Both sample types generated genotypes of sufficient quality for individual identification and the total population size was estimated to be 37 moose, 95% CI [30, 52]. Average amplification rates of twig samples identified as moose and fecal samples were 0.81 and 0.61, respectively. However, genotyping error rates were relatively high in both sample types and only 10% of twig samples and 35% of fecal samples could be used in population genetic analyses. Amplification rate was not useful for filtering out samples with a high error rate, since some samples displayed high error rates despite 100% amplification. We found that graphical analysis of the distribution of allelic differences between all samples is an efficient way of separating real genetic variation from genotyping errors and for deciding the rate of genotyping errors that can be tolerated when grouping genotypes for individual identification.

Keywords *Alces Alces* · Non-invasive sampling · SNP genotyping · Population estimation · Mark-recapture

Introduction

Reliable population size estimates are fundamental for successful management and conservation of wildlife species (Mills 2012; Rosenberg et al. 1995). The last few decades have seen the rapid advancement of DNA techniques granting researchers and managers new tools for estimating abundance and gaining insights into genetic parameters such as connectivity, hybridization and inbreeding (Hohenlohe et al. 2021; Taberlet et al. 1999). Non-invasive genetic mark-recapture has emerged as an alternative to traditional methods involving trapping or sedation of animals which can lead to stress or additional mortality (Soulsbury et al. 2020), and biases resulting from trap responses (Hwang and Huggins 2005). Additionally, non-invasive mark-recapture is generally more time and cost effective and allows for the collection of larger sample sizes (Carroll et al. 2018; Miller et al. 2005).

However, non-invasive genetic sampling comes with its own set of challenges. The DNA that animals leave behind in the environment is usually found in small quantities and the quality rapidly declines with time as it is exposed to precipitation, UV light, enzymes, etc. In addition, the samples tend to contain PCR inhibitors which prevent DNA amplification (Creel et al. 2003, Lampa et al. 2013; Regnaut et al. 2006). These factors increase the prevalence of genotyping errors that can be especially problematic in genetic mark-recapture studies because erroneous genotypes can lead to a large overestimation

✉ Julia L. Jansson
julia.jansson@slu.se

[1] Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå 901 83, SE, Sweden

[2] Ecology and Environmental Science, Umeå University, Umeå 901 87, SE, Sweden

Published online: 03 September 2025

of the population size (Creel et al. 2003; Taberlet et al. 1999). These errors need to be identified and accounted for when analysing data derived from non-invasively collected samples to achieve a reliable estimation of population size (Lampa et al. 2013).

In this study we collected two types of non-invasive samples, feces and browsed twigs, to estimate the size of a Swedish moose (*Alces alces*) population. Combining two sources of DNA can increase the number of usable samples when it is difficult to find enough from one source, as well as mitigate the impact of heterogeneity associated with one method alone (Boulanger et al. 2008). Fecal samples are a well-tested source of DNA in non-invasive genetic studies for a multitude of species, including moose (Blåhed et al. 2019; Broquet and Petit 2004; Carroll et al. 2018). Saliva is a less commonly used source of non-invasively collected DNA. However, saliva left on plant material has been used to collect DNA from primates (Aylward et al. 2018; Ishizuka et al. 2019), salivary DNA left on prey is sometimes used for the identification of predators (Blejwas et al. 2006; Caniglia et al. 2013; Piaggio et al. 2020) and together with fecal DNA, has been used to estimate the size of a brown bear (*Ursus arctos*) population (Wheat et al. 2016). Saliva left on browsed twigs has previously been used for species identification in ungulates (Nichols et al. 2015; van Beeck Calkoen et al. 2019), but not for individual identification, population size estimation, or for population genetic analyses. Our aim was to investigate whether DNA from browsed twigs could generate genotypes of sufficient quality to be used for these applications as well.

From being near extinction in the 1800 s, the Swedish moose population is now the densest in the world (Wallgren 2023). However, after a large population boom in the 1970 s to ‘80s the population has decreased significantly as a result of intense management. About one fourth of the moose population is harvested each year to limit browsing damage to forestry (SLU Artdatabanken 2024). Accurate population size assessments and knowledge about the population structure are necessary to support the sustainable management of the species (Charlier et al. 2008; Dussex et al. 2023). This study was conducted on two neighbouring plateau mountains in southwestern Sweden that support a semi-isolated moose population. The mountains are colloquially referred to as “the moose mountains” and have a history as royal hunting grounds dating back to 1351 (Nunstedt 2006). The moose population has declined over the last decades, and an evaluation conducted in 2001–2009 pointed to a population with low fitness; low birth weights, decreased ovulation and poor antler development (Svensk Naturförvaltning 2010). Pellet counts are carried out annually in the area, but these are notoriously difficult to generate absolute numbers from (Rönnegård et al. 2008), aerial surveys have been unsuccessful due to weather conditions and low visibility, and hence, there is a demand for corroboration from an additional source of information. Furthermore, we were interested in whether this population is genetically isolated from the surrounding area contributing to the population’s low fitness.

Method

Study site

The study was conducted on two adjacent low plateau mountains called Halleberg (2500 ha) and Hunneberg (4300 ha), together referred to as Halle-Hunneberg, in Västergötland, Sweden (Fig. 1). The mountains are separated by a c. 500 m wide ravine and an average elevation of c. 90 m above the central Swedish lowland, in the hemiboreal zone of south-west Sweden. The landscapes on both plateaus consist largely of productive forests dominated by Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*) with an intermixture of deciduous trees such as pedunculate oak (*Quercus robur*) and European beech (*Fagus sylvatica*). A border of weather-beaten pines and heavily browsed sessile oak (*Quercus petraea*) characterize the periphery and the rocky sides of the two mountains. There are four deer species in the area: moose, red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*).

Sample collection

To study the ungulate population we collected twig and fecal samples simultaneously, before bud break in late April and early May of 2019. We walked along the perimeter of 22 square transects, each 4 km in length, some shorter due to landscape features or the boundary of the study area, in total 66.2 km (Fig. 1). For twig samples, every pine and oak with branches lower than two meters, within a three meter radius from the transect, was inspected for browsed branches. About two centimetres of twig were collected from each bite site with a clipper that was flame sterilized with a butane torch between each sample. In the cases where a tree had been browsed more than once, only one twig was collected per tree to avoid sampling the same browser from the same browsing event multiple times. In total, 293 twig samples were collected, 230 from Hunneberg and 63 from Halleberg, 136 samples from oak and 157 from pine. Twigs were stored

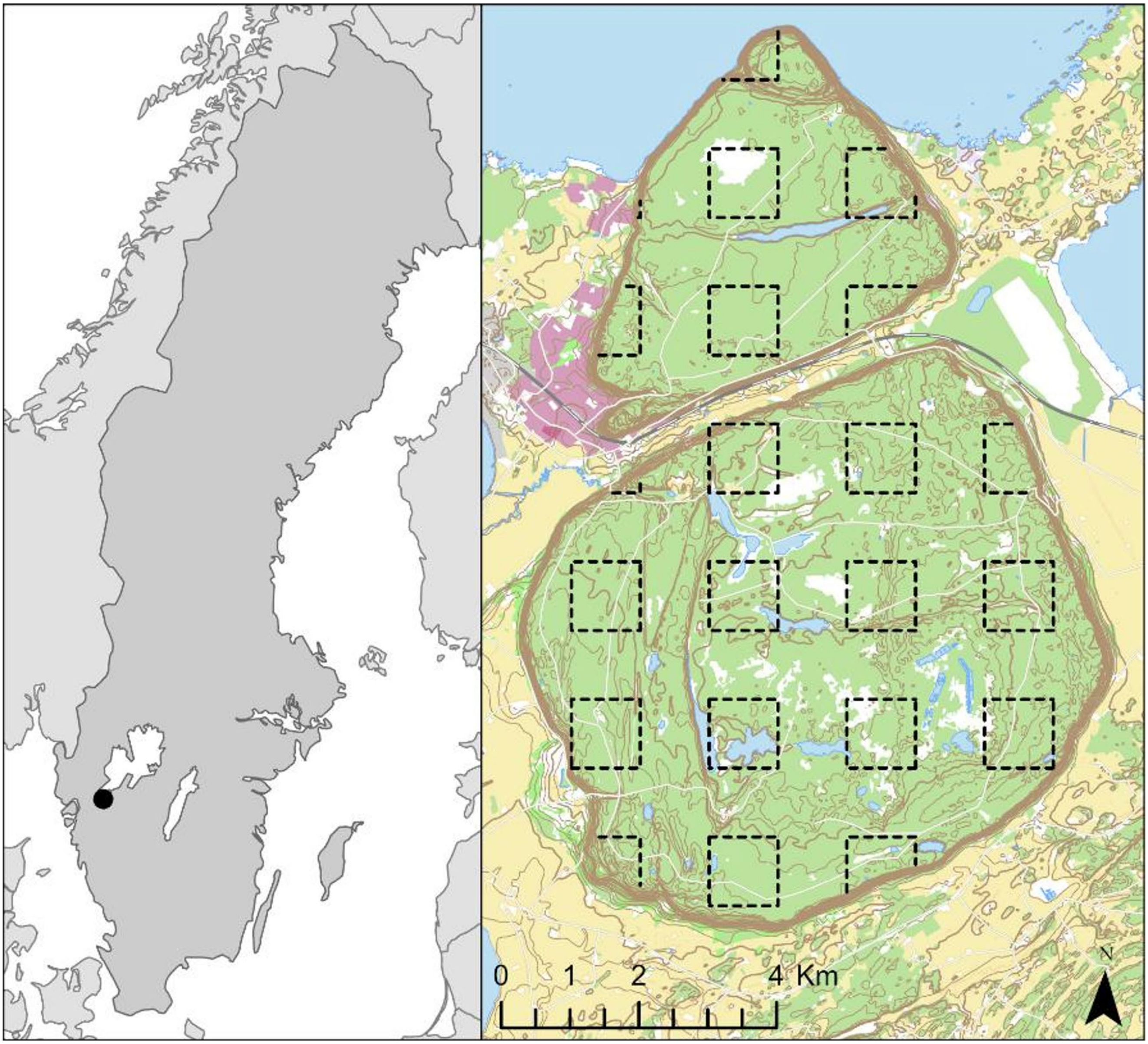

Fig. 1 Left: Map of Sweden showing the location of Halleberg and Hunneberg. Right: Terrain map of Halleberg (north) and Hunneberg (south) with transects shown as dashed lines

individually in kraft paper envelopes in snap-lid containers with silica gel beads to keep them dry. For fecal samples, several pellets from each encountered pile of moose droppings were swabbed with a forensic cotton swab and placed in a tube with a ventilation membrane. Additionally, 2–3 whole pellets were collected in a 50 ml falcon tube containing silica gel beads. In total, swabs and pellets were collected from 97 piles of moose droppings, 57 from Hunneberg and 40 from Halleberg. All samples were stored at room temperature until they could be frozen and stored in the laboratory at −20 °C. Both twig and fecal samples were collected only if they were deemed to have been produced recently enough to generate results in further analyses. Moose droppings were collected if they were dark brown and glossy but not if they were dry or covered in mould. Twigs were judged by the colour of the wood at the surface of the bite, green to white or yellow twigs were collected but not those where the surface of the bite had turned grey. Pine twigs were also judged by the condition of the resin.

DNA extraction

Twigs

DNA was extracted from the twigs using Macherey-Nagel's Nucleospin Soil kit according to manufacturer's instructions with accommodations for using a twig sample instead of soil. The twigs were removed from the tubes after adding the lysis buffer, vortexing for 5 min with ceramic beads, and centrifuging for 2 min. The kit provides two lysis buffers (S1 and S2) of which one is to be chosen depending on the chemical properties of the sample. Additionally, an enhancer (Enhancer SX) is provided that increases DNA yield but may facilitate the release of humic acids that decrease the purity of the DNA. Preliminary tests were run on confirmed bites of moose on twig samples collected from the zoo, Lycksele Djurpark, and evaluated visually with an agarose gel. The best results were obtained using lysis buffer S1 and no Enhancer SX, and this method was thus used for all twig samples.

Fecal samples

The Zymo Research Quick-DNA Fecal/Soil Microbe kit was used to extract DNA from the fecal samples according to manufacturer's instructions. DNA was extracted from both fecal swabs and whole fecal pellets. The DNA on the 97 swabs was extracted by cutting the swab ends into the extraction kit tubes.

Because all whole fecal pellet samples had been swabbed, we extracted DNA from fewer than 97 whole pellets to optimize costs. To compare genotyping results between swab and whole fecal pellet extraction, we extracted DNA from 14 whole fecal pellets whose corresponding swab amplification rates were above 75% in the genotyping stage. To increase the total number of usable moose whole fecal samples in the study, we extracted DNA from 15 additional fecal pellets whose corresponding swab amplification rates were lower than 75%. To optimize DNA quality, only the surface of the fecal pellets was scraped off with a scalpel and used for DNA extraction (as in Blåhed et al. 2019).

SNP genotyping

Twigs

To identify which of the twigs ($n=293$) had been browsed by moose as opposed to other deer species, genotyping was first performed with a SNP assay developed to identify Swedish cervids with 9 mitochondrial SNPs for species identification and between 12 and 26 autosomal SNPs for individual identification depending on the species (for details see Nichols and Spong 2017). All genotyping was performed on the Fluidigm Biomark platform (Fluidigm Corporation, San Francisco, USA). We identified 163 moose genotypes. The amplification rate of each sample was calculated by dividing the number of loci successfully amplified by the total number of loci tested. The 93 samples (a full plate) with the highest amplification rates were then selected for genotyping at four sex-specific and 92 autosomal SNPs allowing for higher precision in individual identification (Blåhed et al. 2018 with adjustments). The SNPs used for sex identification are only amplified in males; if more than two of the four SNPs amplified, the DNA was considered to originate from a male; if two amplified it was deemed inconclusive and if one or none of the SNPs amplified, the sample was considered female. After the initial filtering and validation of the data (see *Validation* below) it was decided that replicates of all samples used in further analyses were needed to calculate the error rate for each sample. Therefore, twig samples with an amplification rate of over 75% of loci ($n=66$) were genotyped a second time. All genotyping runs included three non-template controls (NTCs), containing only water as negative contamination controls and for detecting primer-dimers. Additionally, a sample of moose DNA extracted from tissue sourced from a previous study was included as a positive control in every run.

Fecal samples

The DNA from the fecal swabs was genotyped with the same SNP assay of 92 autosomal moose SNPs as the twigs. The swab DNA samples with amplification success over 75% of loci ($n=49$) were genotyped a second time for replication just as the twig DNA samples. The 14 DNA samples extracted from the fecal pellets with a corresponding swab DNA sample with a > 75% amplification were genotyped once as their error rate could be calculated from comparison with the two replicates of the corresponding swab DNA samples. The 15 whole fecal pellet DNA samples where the corresponding swab had an amplification rate lower than 75% were genotyped twice to be able to calculate error rates.

Genetic analyses

Validation

Amplification rate is typically regarded as a useful proxy for sample quality (Purfield et al. 2016; von Thaden et al. 2017). Thus, only those samples with an amplification rate over 90% were used in the analyses initially. To investigate whether this filtering was sufficient, all sample genotypes were compared pairwise against all sample genotypes, and a graph of the number of allelic differences against the

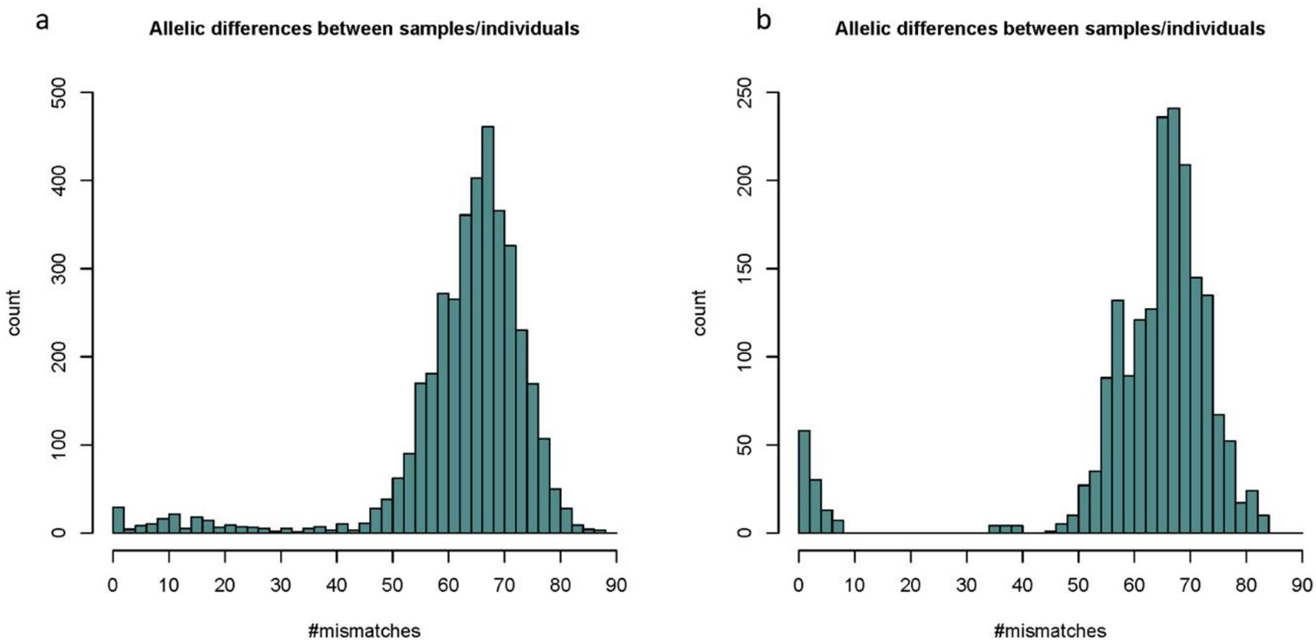

Fig. 2 (a and b). a) Distribution of allelic differences between all samples with >90% amplification rate paired against each other in the dataset, where pairs may come from the same individual or from two individuals. In the left part of the histogram b), more samples of low quality, i.e. those with >15% mismatches per locus, have been filtered out, causing clear separation between distribution caused by genotyping error and the larger distribution of comparisons between different individuals

number of paired samples with that number of differences was created using R Statistical Software (v4.2.2; R Core Team 2022; Fig. 2a). This graph displays both the distribution of natural genetic variation between individuals and sample differences within individuals due to genotyping errors. The sample pairs that originate from the same individual will, ideally, have few differences and appear on the left side of the graph while sample pairs from different individuals will have many differences and appear on the right. When filtering was based solely on amplification rate, there was an overlap between the two distributions making it impossible to distinguish between unique individuals and erroneous genotypes, which may lead to over- or underestimation of the true number of individuals in the sample set. To mitigate this risk, we went back and replicated all samples in the genotyping stage. The two replicates of each sample were then compared and the number of mismatching loci between them was divided by the number of loci where an error could have been detected to calculate the per locus error rate of each sample. Samples were then filtered for error rates lower than 0.15 mismatches per locus. Additionally, two loci with an error rate above 0.15 across samples were excluded. Another graph comparing the allelic differences of all filtered samples was produced by comparing the paired samples. There was no longer an overlap between the genetic variation in the samples compared to the variation generated by genotyping errors (Fig. 2b). After visual examination of the graph, it was estimated that a slightly higher number of errors, lower than 0.2 mismatches per loci, would not interfere with individual identification and increase the number of usable samples ($n=62$). The replicates of the chosen samples were then compared and one consensus genotype for each sample was defined as suggested in Pompanon et al. (2005).

To further examine the relationship between the amplification rate and the error rate, a negative binomial general linear model (GLM) was performed with the R software package MASS (Venables and Ripley 2002). Additionally, a Wilcoxon signed rank test was used to compare the amplification rates of the fecal samples that were swabbed in the field to whole fecal pellets scraped in the lab.

Individual identification

The R package allelematch (Galpern et al. 2012) was used to group all samples with DNA originating from the same individual. The sex associated with each sample was included as an added single locus as suggested in the package Supplementary documentation (Galpern et al. 2012). The function amUniqueProfile in allelematch was used to examine the optimal number of mismatching alleles allowed in a group (Fig. 3). When the number of “multiple

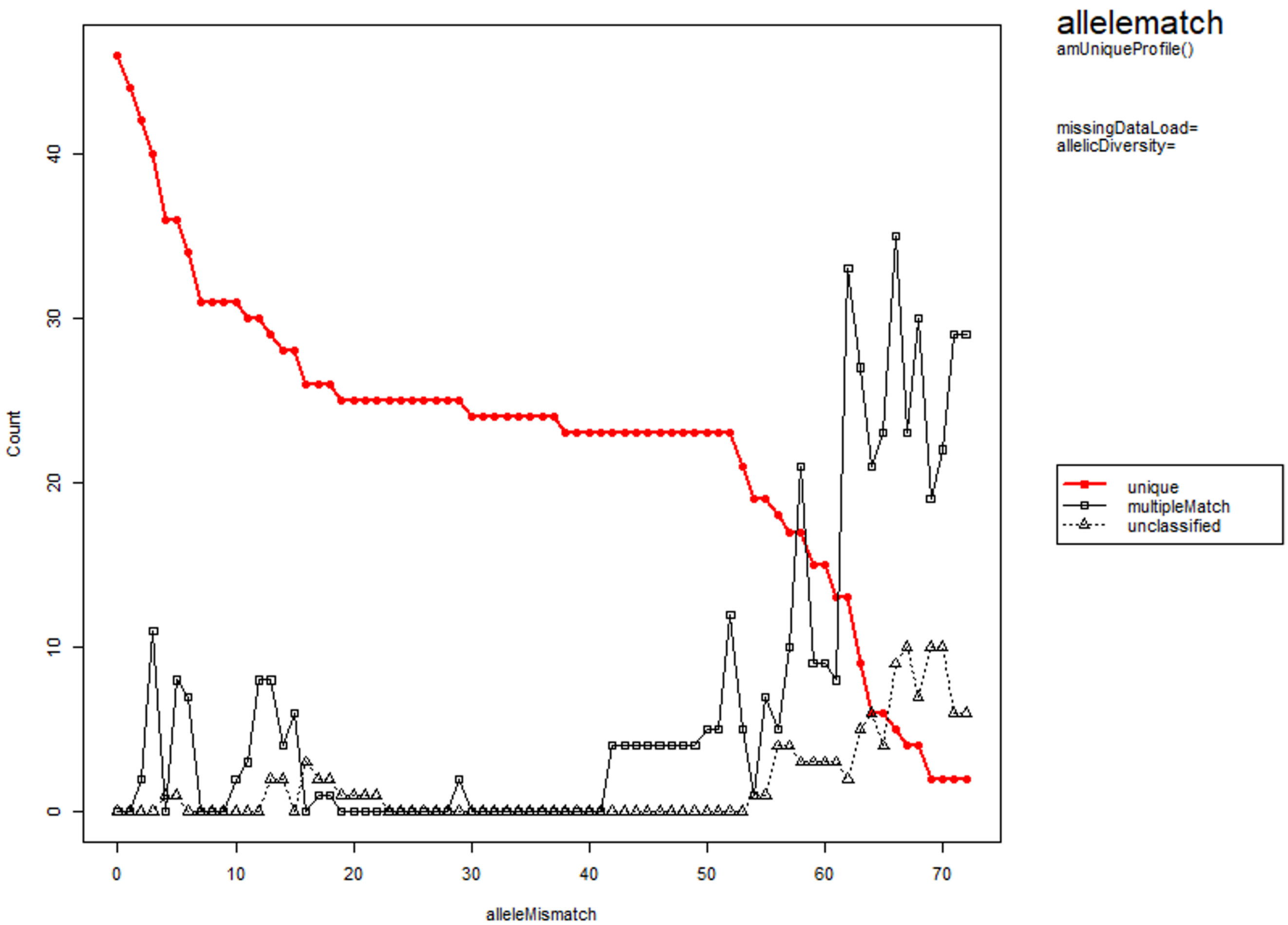

Fig. 3 The output from the function amUniqueProfile in allelematch used to examine the optimal number of mismatching alleles allowed in a group. Count of genotypes identified as unique (unique), genotypes that match several groups (multipleMatch), and number of genotypes unable to be classified to any group (unclassified) against the number of mismatching alleles allowed in a group (alleleMistmatch). When Mutliplematch approaches zero it indicates that genotypes have been sorted into groups with minimal overlap

match" samples (samples that match several groups) first reaches a minimum it indicates that unique genotypes are sorting into groups with minimal overlap (Galpern et al. 2012). The samples had been filtered for a specific known maximum error rate of 0.2 or 18 of 90 (two loci were excluded because of a high error rate across samples) mismatching loci or about 18 of 180 alleles. Thus, a minimum of multiple matches close to that at 19, was investigated initially. The function amUnique was then used to identify individuals in the data with 19 as the input for allowed allelic mismatches. This resulted in one sample being labeled as "unclassified". This means the sample was not different enough to be labelled as a unique individual but it had too many differences to join any of the groups. The amUnique function was run again with 23 as the number of allowed mismatches as that was where the unclassified sample joined a group. The allelic differences between the sample and the group that the sample joined were studied and the mismatches largely consisted of missing data; 2 actual mismatches and 10 missing alleles, which allelematch still counts as an allele mismatch. There were no other changes compared to using 19 as the cut off, other than the unclassified sample joining a group. The next two changes in the number of groups identified when an increasing number of mismatches were allowed, were also investigated. With 30 mismatches allowed, two samples previously identified as unique formed a group of two with 7 mismatches and 22 cases of missing alleles across the two samples. Seven was within the expected number of mismatches due to errors so this group was deemed valid. The next change in unique genotypes at 38 mismatching alleles resulted in two groups of four and two to merge.

Here the new group was deemed invalid as there were 36 actual allelic mismatches and only two cases of missing data. It was also known from the graph of allelic differences (Fig. 2b) that the real variation between individuals started somewhere around 35 allelic differences. Thus, 30 mismatching alleles was chosen as the optimum, noting that only two changes resulting from missing data actually differed from the result with 19 mismatches allowed.

Population size estimation

The R package capwire (Pennell et al. 2013) was used to estimate the population size in R version 3.4.0 (R Core Team 2017). Capwire was developed for estimating population size using mark-recapture from non-invasive genetic samples where, in contrast to traditional capture-mark-recapture, there is often only one capture session where individuals may be caught multiple times (Pennell et al. 2013). Additionally, it has been shown to produce accurate population estimates in small populations with large capture heterogeneity (Miller et al. 2005). The population size was estimated for the whole study area, i.e. both Halleberg and Hunneberg. Two models were fit to the data: the Equal Capture Model (ECM), in which all individuals are assumed to have the same probability of being captured, and the Two-Innate Rates Model (TIRM), in which the population is assumed to contain a mixture of two classes: individuals that are easy to capture and individuals that are difficult to capture. The models were compared using the likelihood ratio test in capwire. Additionally, a 95% confidence interval of the population estimate was calculated using parametric bootstrapping in capwire.

Population genetic analyses

Tests for Probability of identity (PI), Hardy-Weinberg equilibrium (HWE) and mean observed and expected heterozygosity were carried out in GenAlEx 6.503 (Peakall and Smouse 2006, 2012) using the genotypes identified as unique in allelematch. To investigate if the moose population on the mountains differs genetically from the moose in the surrounding areas, genotypes from a previous study were used as a reference group (Blåhed et al. 2019). The moose population in Sweden is primarily divided into two genetic clusters, with one group located in the north and the other in the south (Blåhed et al. 2019; Charlier et al. 2008; Wennerström et al. 2016). Representing the southern cluster, 29 genotypes from moose originating from Uppsala, Öster-Malma, Mark, Aneby, Misterhult, and Växjö were selected. The northern cluster was represented by 28 genotypes of moose originating from Abisko, Nikkaluokta, Överkalix, Arjeplog, Ängesbyn, Hemavan, Malå, Bågede and Nordmaling. A principal coordinate analysis (PCoA) using the genotypes from Halle-Hunneberg and southern and northern Sweden was carried out in GenAlEx to examine the genetic distance between the groups. Additionally, a pairwise F_{st} value, or fixation index, was calculated between Halle-Hunneberg and southern Sweden in GenAlEx using an analysis of molecular variance (AMOVA) approach, with 999 as the number of permutations for the test of significance. The inbreeding coefficient (F) was calculated for each individual in the R package adegenet (Jombart 2008; Jombart and Ahmed 2011).

Results

Genotyping evaluation

The mitochondrial SNPs for species identification from the 293 twig samples amplified at an average rate of 0.76 on the cervid SNP assay with 163 samples identified as moose, 18 as red deer and 8 as roe deer. The twig samples that were identified as carrying moose DNA and were selected for further genotyping ($n = 93$) had an average amplification rate of 0.81 on the moose SNP assay. The average amplification rate of the fecal samples was 0.61 for the swab samples ($n = 97$) and for whole feces it was 0.75 ($n = 29$). There were no significant differences in the amplification rates between fecal samples swabbed in the field and those where a fecal pellet had been collected from the same pile of moose droppings ($n = 29$, Wilcoxon signed rank test, $p > 0.2$). After quality filtering, the data consisted of 62 samples (28 twig samples and 34 fecal samples) with an average amplification rate of 0.98 (range 0.85-1) and a mean error rate of 0.07 (SD = 0.07, range 0-0.19) per locus. Two loci with an error rate above 0.15 across samples were excluded, resulting in 90 autosomal SNPs used in all further analyses. Using only the samples with unique genotypes identified in allelematch ($n = 24$), seven of the SNPs deviated from HWE ($p < 0.05$) and the mean minor allele frequency (MAF) was estimated to be 0.37. The probability of identity (PI) was estimated below 0.001 ($p = 9.97 \cdot 10^{-4}$) combining eight of the most informative SNPs and 15 SNPs among siblings ($PI_{sib,}$ $p = 8.46 \cdot 10^{-4}$).

Although the genotyping error rate decreased slightly with the amplification rate (glm, x2 = 11.23 $p = 8.05e^{-4}$), the variation in error rates was too high even in samples with high amplification rates for it to be a useful proxy for sample quality. Samples with an amplification rate higher than 0.9 had an error rate ranging between 0 and 0.47 (mean = 0.14, SD = 0.12), and one third of those samples had an error rate over 0.2. Thus, amplification rate was determined to not

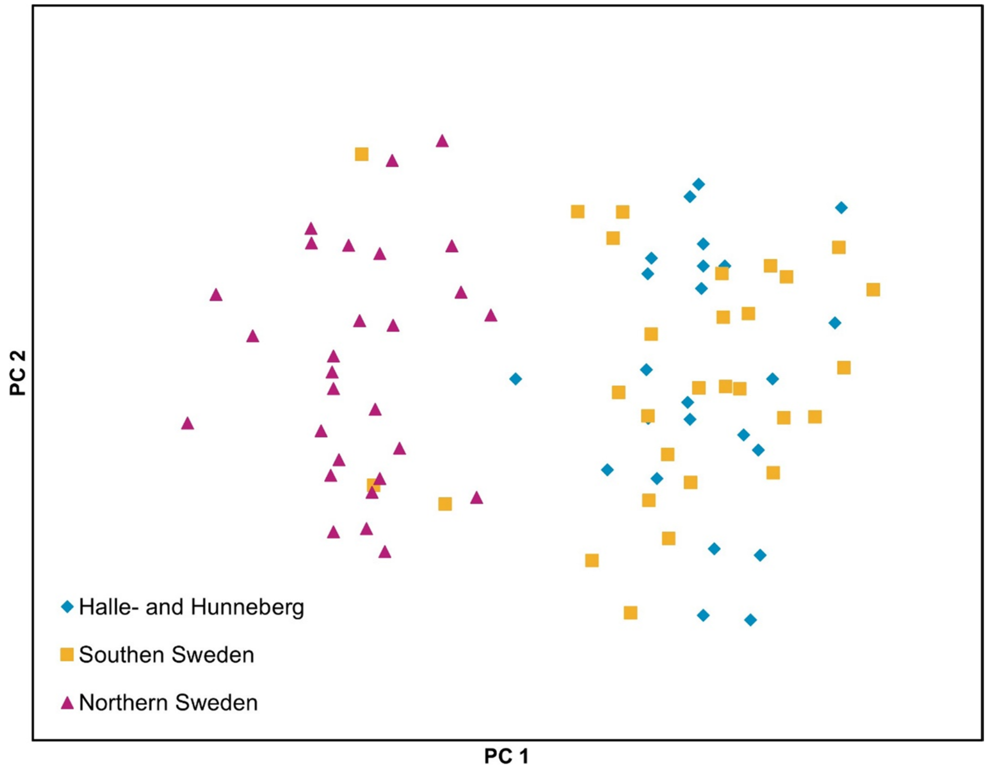

Fig. 4 PCoA visualising population differentiation between moose on Halle-Hunneberg southern Sweden. PC 1, 2 and 3 explain 9.19%, 4.73% and 4.26% of the variation, respectively

be useful for filtering out samples with low quality in this dataset in contrast to findings from earlier studies (Purfield et al. 2016; von Thaden et al. 2017).

Individual identification

Allelematch identified 24 unique individuals in the data, 12 genotypes were observed more than once, up to eight times, and 12 were only observed once (average: 2.6). Ten of the individuals were detected in twig samples, 19 in fecal samples, and five were detected in both types of samples. Of the 24 unique individuals, 7 (29%) were female and 17 (71%) male, five of the individuals were from samples collected on Halleberg and 19 from Hunneberg, no individual was found on both mountains. There were no unclassified samples or samples matching multiple groups with 30 as the number of maximum allele mismatches allowed.

Population size estimation

The result of the likelihood ratio test in capwire was that the null-model, that there is no within population heterogeneity in the probability of capture (ECM), could be rejected in favour of the model with heterogeneity (TIRM test stat: 20.3, $p<0.05$). The population size estimate using the TIRM was 37 individuals 95% CI [30, 52].

Population genetic analyses

The mean observed and expected heterozygosity were 0.43 ± 0.01 (SE) and 0.45 ± 0.01 (SE), respectively, in the Halle-Hunneberg population. For the samples from southern Sweden, the mean observed and expected heterozygosity were 0.44 ± 0.01 and 0.46 ± 0.01, respectively. No significant differences in observed or expected heterozygosity were found between the populations with the z-test (H_{obs} $p=0.59$, H_{exp} $p=0.48$). The F_{st} (fixation index) between Halle-Hunneberg and southern Sweden was not significantly different from zero ($F_{st}=0.01$ $p=0.05$) and the average inbreeding coefficient (F) was 0.52. The PCoA comparing the genetic distance between Halle-Hunneberg, southern and northern Sweden showed high similarity between Halle-Hunneberg and southern Sweden indicating that there is a high level of connectivity between those groups (Fig. 4).

Discussion

Non-invasive samples

In this study we combined two types of non-invasively collected DNA, feces and, for the first time, browsed twigs, to estimate the size of a moose population. The DNA in saliva left on twigs has previously been used for species identification in cervids (Nichols et al. 2012, 2015; Nichols and Spong 2014; van Beeck Calkoen et al. 2019). Here we demonstrated that browsed twigs can also be used for individual identification, to determine population size and investigate population structure. The DNA on twig samples had a high average amplification rate compared to fecal samples on the moose assay, 0.81 vs. 0.61, however, note that twig samples were preselected for their amplification on the cervid assay. Ultimately, only a small portion of the twig samples could be used for individual identification, about 10%, compared to fecal samples where about 35% of the samples were usable for individual identification. The number of twig samples was reduced to fit the assay size but the samples that were filtered out were of very low quality and would probably not have contributed to the final data set. Nevertheless, browsed twig samples can be used for individual identification while providing additional information about feeding preferences and composition of browsing species in the area.

Combining two types of non-invasively collected samples can be useful in situations where it is difficult to get enough from one source alone (Boulanger et al. 2008; Croose et al. 2016). In this case, finding enough fresh fecal samples was challenging as the quality deteriorates rapidly and it is difficult to accurately determine freshness in the field. Twig and fecal samples were collected during the same field sessions and the addition of twig samples did not greatly increase the sampling effort. Multiple methods of sampling can also mitigate the impact of heterogeneous capture probabilities associated with any one method, resulting in more precise population estimates (Boulanger et al. 2008).

Fecal samples were collected in two ways, swabs and whole pellets, to ensure maximum quality and quantity of the DNA and to assess which method worked best. There were no differences in DNA quality between the two methods. This may, however, be due to the relatively small sample size as other studies have found swabs to be superior (Bach et al. 2022; Quasim et al. 2018). We also found swabs to be much less cumbersome to work with in the laboratory compared to whole feces that need to be scraped carefully with a scalpel. In addition, swabs were convenient in the field as they are lightweight and take up very little space.

Population size

The moose population on Halle- and Hunneberg has decreased during the last few decades and has shown signs of low fitness (Svensk Naturförvaltning 2010). We wanted to determine the size of the population and examine whether there is a genetic component to the problem. The moose population size was estimated to be 37 (30–52), with a density of 5.44 (4.41–7.65) moose/1000 ha. An estimate based on pellet counts from local hunters the same year was slightly higher, 7.47 moose/1000 ha (Fredrik Stenbacka, unpublished data), but falls within the confidence interval of our estimation. The national average density in moose hunting areas during the same period was 7.1 moose/1000 ha after the hunt and about 8.8 before (Widemo et al. 2022). No moose were hunted in the study area for a couple of years prior to the study.

Population genetics

The Swedish moose population has been shown to be primarily structured into two genetic groups, one in the northern and one in the southern part of the country (Blåhed et al. 2019; Charlier et al. 2008; Wennerström et al. 2016). The population of Halle-Hunneberg seems to have a high level of connectivity with the population of southern Sweden, as indicated by the low pairwise F_{st} value and the high similarity in the PCoA and low inbreeding coefficient (F). Moose in Sweden have been found to have relatively restricted gene flow, with genetic similarity decreasing almost linearly across distance with an average dispersal distance of 3.5–11.1 km (Wennerström et al. 2016). Halle-Hunneberg only measures about 8×13 km across at its widest points and moose are a highly mobile species (Singh et al. 2012), individuals probably frequently move in and out of the area as evidenced by the high genetic similarity with the rest of the southern Swedish population. Thus, the decrease in population size and the deterioration of the physical condition of moose is likely not related to any genetic concerns but rather to other issues such as competition for resources and hunting pressure. The available forage on the mountain has decreased during the last decades (Svensk Naturförvaltning 2010), and although hunting has been limited on the actual mountain, the population is connected to the surrounding areas where the goal has been to decrease moose numbers to mitigate forest damage.

Error rate

The error rate in our study was on the higher end of what has been reported in previous non-invasive genetic studies (Lampa et al. 2013; von Thaden et al. 2020), and some samples displayed very high error rates despite high amplification success, which is often used as a measure of data quality (Purfield et al. 2016; von Thaden et al. 2017).

In microsatellite studies, low quality and quantity of target DNA increase the risk of errors such as allelic dropout and false alleles (Taberlet et al. 1999). The relationship between low-quality DNA samples and genotyping errors is not as well studied in SNP markers. SNPs generally perform better in low quality samples; however, allelic dropout and false alleles can still cause significant issues for non-invasively collected samples (von Thaden 2020). Additionally, herbivore feces and browsed twigs samples both contain plant material which tend to contain strong PCR inhibitors (Peist et al. 2001).

Filtering out error prone samples and accurately calculating the error rates is particularly important when using genotypes for individual identification and population size estimation (Waits and Leberg 2000). A single error in a genotype creates a false "ghost" individual which, even at low error rates, can cause a large overestimation of the number of individuals detected and an underestimation of the individuals recaptured, both of which lead to an inflated population size estimation (Creel et al. 2003). Previously, and common for microsatellite studies, the multiple-tube approach, i.e. genotyping the same sample or individual more than once, was used to improve allele calling in individual genotypes (Taberlet et al. 1999). Replication of all samples significantly increases costs but can be necessary in many non-invasive studies as DNA quality is generally low (von Thaden et al. 2020; López-Bao et al. 2020). Amplification rate has been suggested as a proxy for DNA quality to decide whether only some samples need to be replicated (Laguardia et al. 2021; von Thaden et al. 2017). In this study we found that even samples with 100% amplification success could have an error rate as high as 0.23, making it ineffective for quality filtering. We resolved this by requiring two replicates with an error rate below the 0.2 threshold.

Allelematch, and some other programs for identification of unique multilocus genotypes, are developed to tolerate some errors by allowing an accepted number of mismatches between the multilocus genotypes originating from the same individual to be set (Galpern et al. 2012). The allowed number of mismatches is important to get right as being too lenient will result in identifying too few unique genotypes and being too stringent will result in identifying too many, i.e. creating ghost individuals. Investigating the variation in allelic differences in the data graphically (Fig. 2) allowed us to identify that there were too many errors in our initial data set to separate differences caused by errors and actual genetic differences. After more rigorous quality filtering we could determine that real individuals had between 35 and 84 allelic differences between them and the number of errors allowed in the data needed to be less than that so that an allowed number of mismatches could be set safely in between. This method allowed us to include as many samples as possible while ensuring that the errors we allowed in the data did not interfere with differentiating individuals. Nevertheless, because Allelematch counts missing data as mismatches, great care has to be taken to review the results.

Conclusions

Salivary DNA left on twigs by browsing can be used to generate genotypes of sufficient quality for individual identification and to study population genetics. However, as with many non-invasive genetic samples, low quality and quantity of target DNA on browsed twigs can inhibit sequencing and cause low amplification and high genotyping error rates. Accounting for these genotyping errors is essential, especially in population size estimation. We found that analysing the distribution of allelic differences between samples graphically is a useful tool for highlighting whether genotyping errors will interfere with individual identification and downstream analyses. If genotypes of sufficient quality can be extracted, however, browsed twigs provide an easy source of non-invasive genetic samples. Twigs were easy to find and collect compared to fecal samples and can provide additional information about browsing preferences and the relative impact of browsing from different species. Whether browsed twigs are a good option for sourcing non-invasively collected genetic samples will depend on the research questions, availability of other sources, such as fecal samples, and the relative cost of field work and sequencing.

Acknowledgements We would like to express our gratitude to Helena Königsson for her expertise and contribution in the laboratory and to Gustav Fält for his field assistance. We are deeply appreciative of the late Jimmy Pettersson for his helpful guidance during field visits. We also thank Sveaskog for granting us permission to conduct this study on Halle- and Hunneberg.

Author contributions JLJ and GS came up with the research question and experimental setup. JLJ collected all data, ran preliminary analyses and wrote the first draft. All authors contributed in interpreting the results and provided input on previous drafts.

Funding Open access funding provided by Swedish University of Agricultural Sciences. This work was funded by a grant by Stiftelsen Skogssällskapet and Gunnar and Lillian Nicholson Graduate Fellowship and Faculty Exchange Fund.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

Aylward ML, Sullivan AP, Perry GH, Johnson SE, Louis EE (2018) An environmental DNA sampling method for aye-ayes from their feeding traces. Ecol Evol 8(18):9229–9240. https://doi.org/10.1002/ece3.4341

Bach BH, Quigley AB, Gaynor KM, McInturff A, Charles KL, Dorcy J, Brashares JS (2022) Identifying individual ungulates from fecal DNA: a comparison of field collection methods to maximize efficiency, ease, and success. Mamm Biol 102(3):863–874. https://doi.org/10.1007/s42991-021-00176-5

Blåhed IM, Königsson H, Ericsson G, Spong G (2018) Discovery of SNPs for individual identification by reduced representation sequencing of moose (*Alces alces*). PLoS One 13(5):e0197364

Blåhed IM, Ericsson G, Spong G (2019) Noninvasive population assessment of moose (*Alces alces*) by SNP genotyping of fecal pellets. Eur J Wildl Res 65(6):96

Blejwas KM, Williams CL, Shin GT, McCullough DR, Jaeger MM (2006) Salivary DNA evidence convicts breeding male Coyotes of killing sheep. J Wildl Manage 70(4):1087–1093. https://doi.org/10.2193/0022-541x(2006)70[1087:sdecbm]2.0.co;2

Boulanger J, Kendall KC, Stetz JB, Roon DA, Waits LP, Paetkau D (2008) Multiple data sources improve DNA-based mark recapture population estimates of grizzly bears. Ecol Appl 18(3):577–589. https://doi.org/10.1890/06-1941.1

Broquet T, Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. Mol Ecol 13(11):3601–3608. https://doi.org/10.1111/j.1365-294X.2004.02352.x

Caniglia R, Fabbri E, Mastrogiuseppe L, Randi E (2013) Who is who? Identification of livestock predators using forensic genetic approaches. Forensic Sci Int Genet 7(3):397–404. https://doi.org/10.1016/j.fsigen.2012.11.001

Carroll EL, Bruford MW, DeWoody JA, Leroy G, Strand A, Waits L, Wang J (2018) Genetic and genomic monitoring with minimally invasive sampling methods. Evol Appl 11(7):1094–1119. https://doi.org/10.1111/eva.12600

Charlier J, Laikre L, Ryman N (2008) Genetic structure and evidence of a local bottleneck in moose in Sweden. J Wildl Manag 72(2):411–415

Creel S, Spong G, Sands JL, Rotella J, Zeigle J, Joe L, Murphy KM, Smith D (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Mol Ecol 12(7):2003–2009. https://doi.org/10.1046/j.1365-294X.2003.01868.x

Croose E, Birks JDS, O'Reilly C, Turner P, Martin J, MacLeod ET (2016) Sample diversity adds value to non-invasive genetic assessment of a pine Marten (*Martes martes*) population in Galloway forest, Southwest Scotland. Mammal Res 61(2):131–139. https://doi.org/10.1007/s13364-015-0257-6

Dussex N, Kurland S, Olsen RA, Spong G, Ericsson G, Ekblom R, Laikre L (2023) Range-wide and temporal genomic analyses reveal the consequences of near-extinction in Swedish moose. Commun Biol 6(1):1035

Galpern P, Manseau M, Hettinga P, Smith K, Wilson P (2012) Allelematch: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. Mol Ecol Resour 12:771–778

Hohenlohe PA, Funk WC, Rajora OP (2021) Population genomics for wildlife conservation and management. Mol Ecol 30(1):62–82

Hwang WH, Huggins R (2005) An examination of the effect of heterogeneity on the estimation of population size using capture-recapture data. Biometrika 92(1):229–233

Ishizuka S, Kawamoto Y, Toda K, Furuichi T (2019) Bonobos' saliva remaining on the pith of terrestrial herbaceous vegetation can serve as non-invasive wild genetic resources. Primates 60(1):7–13. https://doi.org/10.1007/s10329-018-00704-x

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart T, Ahmed I (2011) Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics. https://doi.org/10.1093/bioinformatics/btr521

Laguardia A, Bourgeois S, Strindberg S, Gobush KS, Abitsi G, Ateme HBB, Stokes EJ (2021) Nationwide abundance and distribution of African forest elephants across Gabon using non-invasive SNP genotyping. Glob Ecol Conserv 32:e01894

Lampa S, Henle K, Klenke R, Hoehn M, Gruber B (2013) How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation - a review of available methods illustrated by a case study. J Wildl Manage 77:1490–1511. https://doi.org/10.1002/jwmg.604

López-Bao Jv, Godinho R, Rocha RG, Palomero G, Blanco JC, Ballesteros F, Jiménez J (2020) Consistent bear population DNA-based estimates regardless molecular markers type. Biol Conserv. https://doi.org/10.1016/j.biocon.2020.108651

Miller CR, Joyce P, Waits LP (2005) A new method for estimating the size of small populations from genetic mark–recapture data. Mol Ecol 14:1991–2005

Mills LS (2012) Conservation of wildlife populations: demography, genetics, and management. Wiley

Nichols Rv, Spong G (2014) Ungulate browsing on conifers during summer as revealed by DNA. Scand J for Res 29(7):650–652. https://doi.org/10.1080/02827581.2014.960895

Nichols Rv, Spong G (2017) An edna-based SNP assay for ungulate species and sex identification. Diversity 9(3):33

Nichols Rv, Königsson H, Danell K, Spong G (2012) Browsed twig environmental DNA: diagnostic PCR to identify ungulate species. Mol Ecol Resour 12(6):983–989. https://doi.org/10.1111/j.1755-0998.2012.03172.x

Nichols Rv, Cromsigt JPGM, Spong G (2015) Using edna to experimentally test ungulate browsing preferences. SpringerPlus. https://doi.org/10.1186/s40064-015-1285-z

Nunstedt H (2006) Kungens och Älgarnas berg: Halle- och Hunneberg. Warne Förlag

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Peist R, Honsel D, Twieling G, Löffert D (2001) PCR inhibitors in plant DNA preparations. Qiagen News 3:7–9

Pennell MW, Stansbury CR, Waits LP, Miller CR (2013) Capwire: a R package for estimating population census size from non-invasive genetic sampling. Mol Ecol Resour 13:154–157. https://doi.org/10.1111/1755-0998.12019

Piaggio AJ, Shriner SA, Young JK, Griffin DL, Callahan P, Wostenberg DJ, Gese EM, Hopken MW, Heske E (2020) DNA persistence in predator saliva from multiple species and methods for optimal recovery from depredated carcasses. J Mammal 101(1):298–306. https://doi.org/10.1093/jmammal/gyz156

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. Nat Rev Genet 6(11):847–859

Purfield DC, McClure M, Berry DP (2016) Justification for setting the individual animal genotype call rate threshold at 85%. J Anim Sci 94(11):4558–4569. https://doi.org/10.2527/jas.2016-0802

Quasim S, MacDonald AJ, Sarre SD (2018) Towards more efficient large-scale DNA-based detection of terrestrial mammal predators from scats. Mammal Res 63(3):387–393. https://doi.org/10.1007/s13364-018-0369-x

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Regnaut S, Lucas FS, Fumagalli L (2006) DNA degradation in avian faecal samples and feasibility of non-invasive genetic studies of threatened capercaillie populations. Conserv Genet 7(3):449–453. https://doi.org/10.1007/s10592-005-9023-7

Rönnegård L, Sand H, Andrén H, Månsson J, Pehrson Å (2008) Evaluation of four methods used to estimate population density of moose *Alces alces*. Wildl Biol 14(3):358–371

Rosenberg DK, Overton WS, Anthony RG (1995) Estimation of animal abundance when capture probabilities are low and heterogeneous. J Wildl Manag 59(2):252–261. https://doi.org/10.2307/3808938

Singh NJ, Börger L, Dettki H, Bunnefeld N, Ericsson G (2012) From migration to nomadism: movement variability in a Northern ungulate across its latitudinal range. Ecol Appl 22(7):2007–2020. https://doi.org/10.1890/12-0245.1

SLU Artdatabanken (2024) Artfakta: älg (Alces alces). https://artfakta.se/taxa/206046, last Accessed 12 Nov 2024

Soulsbury C, Gray H, Smith L, Braithwaite V, Cotter S, Elwood RW, Collins LM (2020) The welfare and ethics of research involving wild animals: a primer. Methods Ecol Evol 11(10):1164–1181

Svensk Naturförvaltning AB (2010) Ålderssammansättning, reproduktion och hornutveckling Hos älg, Kronhjort och Rådjur På Halle- och Hunneberg 2001–2009. Rapport 2010-3

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. Trends Ecol Evol 14:323–327

van Beeck Calkoen ST, Leigh-Moy K, Cromsigt JP, Spong G, Lebeau LC, Heurich M (2019) The blame game: using eDNA to identify species-specific tree browsing by red deer (Cervus elaphus) and roe deer (Capreolus capreolus) in a temperate forest. For Ecol Manag 451:117483

Venables WN, Ripley BD (2002) Modern applied statistics with S, Fourth edition. Springer, New York. ISBN 0-387-95457-0. https://www.stats.ox.ac.uk/pub/MASS4/

von Thaden A, Cocchiararo B, Jarausch A, Jüngling H, Karamanlidis AA, Tiesmeyer A, Nowak C, Muñoz-Fuentes V (2017) Assessing SNP genotyping of noninvasively collected wildlife samples using microfluidic arrays. Sci Rep. https://doi.org/10.1038/s41598-017-10647-w

von Thaden A, Nowak C, Tiesmeyer A, Reiners TE, Alves PC, Lyons LA, Mattucci F, Randi E, Cragnolini M, Galián J, Hegyeli Z, Kitchener AC, Lambinet C, Lucas JM, Mölich T, Ramos L, Schockert V, Cocchiararo B (2020) Applying genomic data in wildlife monitoring: development guidelines for genotyping degraded samples with reduced single nucleotide polymorphism panels. Mol Ecol Resour 20(3):662–680. https://doi.org/10.1111/1755-0998.13136

Waits JL, Leberg PL (2000) Biases associated with population estimation using molecular tagging. Anim Conserv 3(3):191–199. https://doi.org/10.1111/j.1469-1795.2000.tb00103.x

Wallgren M (2023) Sveriges älgstam fortfarande i topp. Skogforsk, kunskapsbanken. https://www.skogforsk.se/kunskapsbanken/kunskapsartiklar/2022/sverige-har-varldens-tataste-algstam/. Accessed 13 Nov 2024

Wennerström L, Ryman N, Tison JL, Hasslow A, Dalén L, Laikre L (2016) Genetic landscape with sharp discontinuities shaped by complex demographic history in moose (*Alces alces*). J Mammal 97(1):1–13

Wheat RE, Allen JM, Miller SDL, Wilmers CC, Levi T (2016) Environmental DNA from residual saliva for efficient noninvasive genetic monitoring of brown bears (*Ursus arctos*). PLoS One. https://doi.org/10.1371/journal.pone.0165259

Widemo F, Leonardsson K, Ericsson G (2022) Samförvaltning av älg och skog–analyser av den nya älgförvaltningen under perioden 2012–2021: Co-management of the Swedish moose population and Swedish forests–analyses at the level of moose management areas during 2012–2021. Naturvårdsverket