Scottish Natural Heritage
Research Report No. 910

Can genetic techniques help estimate capercaillie (*Tetrao urogallus*) population size and survival rates – a pilot study to develop survey methods

Scottish Natural Heritage
Dualchas Nàdair na h-Alba
All of nature for all of Scotland
Nàdar air fad airson Alba air fad

RESEARCH REPORT

Research Report No. 910

Can genetic techniques help estimate capercaillie (*Tetrao urogallus*) population size and survival rates – a pilot study to develop survey methods

For further information on this report please contact:

Sue Haysom
Scottish Natural Heritage
Great Glen House
INVERNESS
IV3 8NW
Telephone: 01463 725225
E-mail: susan.haysom@snh.gov.uk

This report should be quoted as:

Fletcher, K., Baines, D., Ghazali, M. & Murray-Dickson, G. 2018. Can genetic techniques help estimate capercaillie (*Tetrao urogallus*) population size and survival rates – a pilot study to develop survey methods. *Scottish Natural Heritage Research Report No. 910.*

RESEARCH REPORT

Summary

Can genetic techniques help estimate capercaillie (*Tetrao urogallus*) population size and survival rates – a pilot study to develop survey methods

Research Report No. 910
Project No: 016420
Contractor: The Game & Wildlife Conservation Trust
Year of publication: 2018

Keywords

Capercaillie; population estimate; survey methods; DNA

Background

Capercaillie *Tetrao urogallus* numbers have declined in Scotland since at least the mid-1970s, (Moss, 1994; Catt *et al*., 1998). The national survey conducted over-winter 2009-2010 estimated the capercaillie population in Scotland to be 1285 individuals (95% CL 822-1822; Ewing *et al.,* 2012). The current distance sampling method being used for Scottish national surveys is labour intensive and produces population estimates with large confidence intervals, so it is difficult to detect significant differences in population size and trends at a local scale.

Genetic techniques have been widely used to examine historical and contemporary distribution of genetic diversity across capercaillie populations in Europe. Furthermore, they have been used to estimate population size and connectivity among local populations (Jacob *et al*., 2010; Morán-Luis *et al.,* 2014; Rösner *et al*., 2014). The feasibility of using genetic techniques to estimate the population size largely depends upon underlying population genetic structure and diversity. Whilst the Scottish population has previously been included in both population genetic and phylogeographic analysis of European populations, (Segelbacher *et al.,* 2003, Segelbacher & Piertney, 2007), the use of genetic profiling to estimate population size has not been previously considered. Moreover, such estimates have not been compared to those gained from current survey methods across the Scottish population. This pilot study aims to determine whether such genetic methods would provide a feasible alternative to those field methods currently applied in Scotland.

Main findings

- A total of 113 capercaillie feather samples were collected within two forests in Strathspey (Kinveachy and Rothiemurchus) in April / May or August 2014. From these, 87 individuals were genotyped using nine microsatellite DNA markers. This indicates that the genetic techniques trialled were successful in distinguishing individuals.
- Among the 87 samples for which all genotype data were available, we detected seven duplicate samples suggesting 80 unique individuals (range of 69 - 82 individuals accounting for the presence of missing genetic data).

- The 'population size' was based on the number of unique multilocus genotype profiles observed in the sample set: 55 in Kinveachy, which included 33 males, and 25 in Rothiemurchus, which included 14 males. This should be considered as the minimum number of individuals, as the low recapture rate meant capture mark recapture (CMR) techniques could not be used to estimate a robust population size.
- The numbers of males and all adults recorded at spring leks were higher than the number detected from genetic analysis on Rothiemurchus (lekking males = 28, all adults recorded at leks = 36), but lower on Kinveachy (lekking males = 28, all adults recorded at leks = 48).
- The number of individuals from summer brood count surveys were similar to those reported from the genetic survey but 13% lower with 48 individuals in Kinveachy and 8% lower with 23 in Rothiemurchus.
- The samples collected for this pilot study primarily involved a single survey with transects walked 100m apart in August. As this sampling design did not yield enough feather samples to allow CMR analyses, it may be necessary to double the intensity of sampling by repeating surveys and / or focus on the collection of both feathers and faecal samples during lek period to estimate the population size for males only.
- As the genetic markers were successful we would expect it to be possible to estimate population size and survival rates between consecutive surveys with more intensive sampling.
- A national survey using genetic techniques may require considerable man-power to collect enough samples. Further consideration is needed to determine how best this could be resourced; whether strategic sample collection for genetic analysis could be combined with other field surveys and / or sampling should focus on smaller geographical areas.

This project was a partnership project between Scottish Natural Heritage, Game & Wildlife Conservation Trust and WildGenes laboratory of the Royal Zoological Society Scotland.

Scottish Natural Heritage
Dualchas Nàdair na h-Alba
All of nature for all of Scotland
Nàdar air fad airson Alba air fad

Game & Wildlife
CONSERVATION TRUST
Scotland

RZSS
BRINGING CONSERVATION TO LIFE

For further information on this project contact:
Sue Haysom, Scottish Natural Heritage, Great Glen House, Inverness, IV3 8NW.
Tel: 01463 725225 or susan.haysom@snh.gov.uk
For further information on the SNH Research & Technical Support Programme contact:
Knowledge & Information Unit, Scottish Natural Heritage, Great Glen House, Inverness, IV3 8NW.
Tel: 01463 725000 or research@snh.gov.uk

Table of Contents **Page**

Acknowledgements

We would like to thank all the forest owners and their agents, foresters and gamekeepers for access permission to complete this survey. Assistance with field sample collection was provided by Sion Thomas, Graeme Neish, Philip Warren and Michael Richardson. Samples from leks were collected by Capercaillie Project Officer and his assistants. Surveys at leks were undertaken by Capercaillie Project Officer, his assistant and staff employed by each estate. The national survey was funded by RSPB and SNH with data collected by staff employed on this project. All laboratory work was undertaken by Jennifer Kaden and Muhammad Ghazali at the WildGenes Laboratory, Royal Zoological Society of Scotland.

1. INTRODUCTION

The capercaillie population in Scotland has been declining since the 1970s (Moss, 1994) to an estimate of 2200 (95% CL 1500 – 3200) individuals in a national survey in 1993/4 (Catt *et al.,* 1998), 1073 individuals (95% CL 549 – 2041) in 1998/9 (Wilkinson *et al.,* 2002) and 1980 individuals (95% CL 1284-2758) in 2003/4 (Eaton *et al.,* 2007). The national survey conducted over-winter 2009-2010 estimated the capercaillie population in Scotland to be 1285 individuals (95% CL 822-1822; Ewing *et al.,* 2012). The current distance sampling method being used for Scottish national surveys produce population estimates with confidence intervals that are on average 46% of the mean (1993/4 CL was 32% and 45% of mean; 1998/9 CL was 49% and 90% of mean; 2003/4 CL was 35% and 39% of mean and 2009/10 CL was 36% and 42% of mean), so it is difficult to detect significant differences in population size. Furthermore, this method is labour intensive and lacks the precision to detect population changes at a local scale.

Annual lek counts are used to assess numbers and population trends of males. Lek surveys may provide accurate local trends if the majority of leks are surveyed during the peak activity period. A multiplier of four times the number of males observed at a lek has been suggested to estimate the total breeding population (Watson & Moss, 2008). This is due to the observation that young and sub-adult males do not attend leks. In a closed population, with 30% annual mortality of males, young males will comprise at least 30% of the population and second year sub-adults at least 21% (30% x 70%) which means approximately 50% of the males do not usually attend leks. An equal sex ratio has also been assumed (Watson & Moss, 2008) however, this multiplier has not been confirmed with field data. The difference between the number of males recorded attending a lek and the total population may vary due to inter-annual differences in breeding success and subsequent variation in the recruitment to the lek.

Genetic surveys using non-invasive sampling have been used to estimate population size of capercaillie elsewhere in Europe (Jacob *et al*., 2010; Morán-Luis *et al.,* 2014; Rösner *et al*., 2014). In Switzerland national surveys were traditionally obtained using lek surveys, but genotyping individual birds recorded a higher population than traditional field surveying (Jacob *et al*., 2010). Microsatellite DNA markers were used to assess population structure across the Bohemian forest in Germany/Czech Republic (Rösner *et al*., 2014), and demographic status of a small population at the edge of the species range in north-west Spain, one now considered an Evolutionary Significant Unit (Rodriguez-Muñoz *et al.,* 2007). Mitochondrial DNA derived from feathers during the EU LIFE project 'Urgent action for Scottish capercaillie' (2000-2007; Piertney, 2004) were used to compare the Scottish population with continental counterparts, to determine which populations should be recognised as subspecies (Segelbacher & Piertney, 2007), however, estimation of population size was not considered. Whilst the utility of genetic surveys has been demonstrated in continental Europe, specific application to the Scottish population requires testing due to potentially different underlying genetic diversity (low genetic diversity would make identifying individuals more problematic).

1.1 Objectives

A. To evaluate the intensity of genetic sampling required to estimate population size.

B. To determine the relationship between local forest population size estimates derived from genetic analysis, to those from cocks attending leks in spring and adults seen during summer brood counts in the same forests.

C. Using results from objectives A and B, assess whether genetic techniques can be a cost effective and accurate means of determining the size of the capercaillie population in Scotland.

2. METHODS

2.1 Collection of field data and samples for genetic analysis

This pilot study focussed on the capercaillie brood counts areas surveyed annually within Kinveachy (11.0km^2) and Rothiemurchus forests (9.7km^2) (Figure 1). Table 1 shows how many samples were collected during each of three surveys. Feathers found within 1m were assumed to be from the same individual and stored as such. Thereafter all samples collected were genetically analysed, with no minimum distance between samples considered.

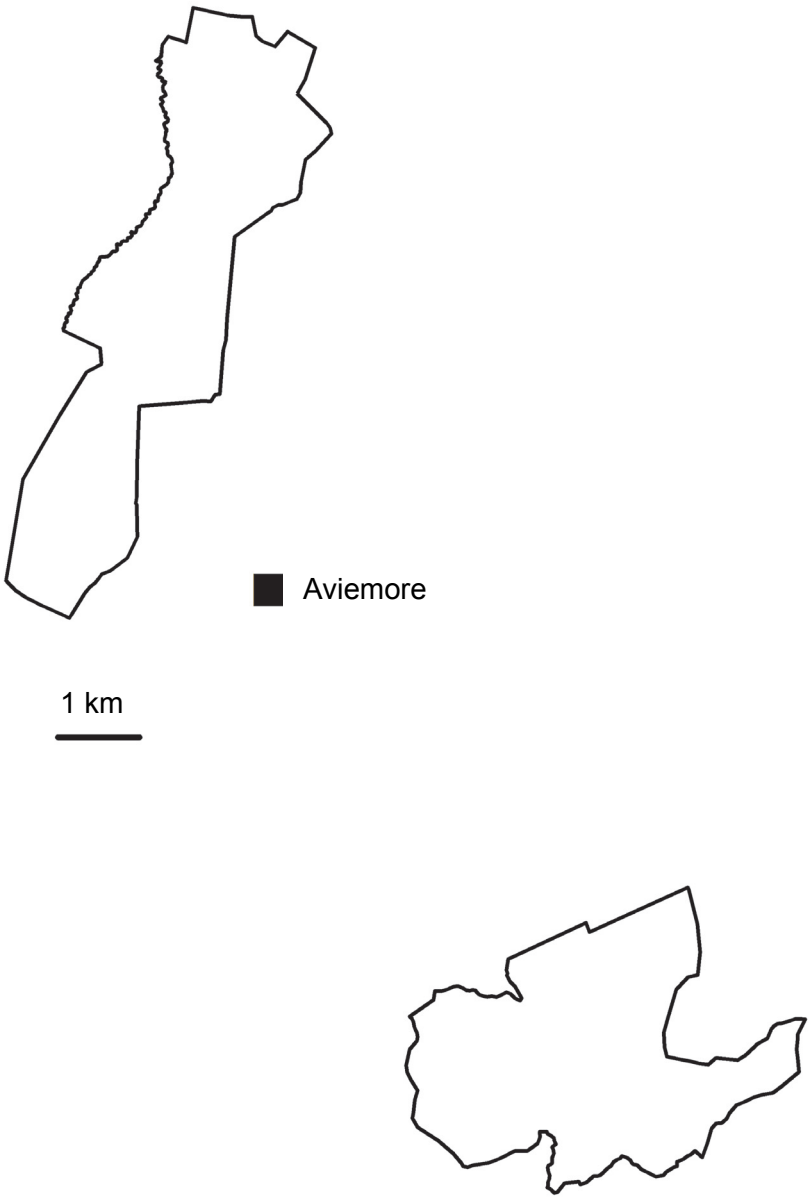

Figure 1. Map of the study area boundaries. Top left = Kinveachy, Bottom right = FES Rothiemurchus © Crown copyright and database right 2016. Ordnance Survey 100017908.

Table 1. The number of capercaillie feather samples collected during three surveys in 2014.

Method	Kinveachy	Rothiemurchus	Total
Lek survey	1	7	8
Spring transect	13	9	22
Brood survey	60	23	83
Total	74	39	113

Individuals at known leks in each forest were counted once in mid- to late-April 2014 when attendance was likely to be highest (SNH, 2013; Watson & Moss, 2008). Surveys were undertaken from hides using between one and three observers on the same day for an individual lek. Surveys were undertaken between 04:00 hrs and 08:00 hrs with each hide at least 10m from the nearest stance, providing the best view of the overall lek area. The number of lekking males was estimated from a combination of sight and sound with confirmation from additional searches for fresh evidence of activity made immediately after the survey. During the searches of the area samples were collected from each known stance. Observers focussed on collecting faecal material although some feathers were also collected.

In addition to the long running spring lek surveys it was also possible to undertake spring transect surveys. The study areas were then overlaid with north-south transects 250m apart (40.0km of transect within Kinveachy and 32.2km within Rothiemurchus). Between 28th April and 16th May 2014 an observer walked these transects recording any capercaillie seen or heard flushing from 10:00 hrs to avoid lekking. To calculate the density of birds from a transect survey a "strip width" is used to estimate the area covered by the survey (Buckland *et al.,* 2001). Frequency histograms of the perpendicular distance of capercaillie from the transect lines walked are published from 2003/4 and 2009/10 winter surveys (Eaton *et al.*, 2007; Ewing *et al*., 2012). From these data the estimated strip widths were 30.4m (95% CI 26.3 – 35.1m, from 105 encounters) and 18.4m (95% CI 14.5 – 23.4m, from 56 encounters) respectively. For this study we used the strip width from the 2003/4 survey where more encounters were recorded resulting in an estimated area of 2.43km^2 (95% CI 2.10 – 2.81km^2) in Kinveachy and 1.96km^2 (95% CI 1.69 – 2.26km^2) in Rothiemurchus. Along these transects a second observer focussed on the ground and collected all feathers seen, recording their location with a hand held GPS.

Surveys of adults and chicks were conducted in August 2014 (Moss *et al.,* 2000). Observers walked transects 50-100m apart, recording birds and in addition collected any feathers, noting the location with a hand held GPS. Transects 100m apart equates to approximately 110km of transects in Kinveachy and 95km in Rothiemurchus. With multiple observers walking neighbouring transects at the same time it is difficult to estimate distance from the central transect line and therefore it has been assumed that the whole study area was covered. Following the genetic analyses (see below) the number of feathers collected per km surveyed was used to estimate how many individuals we predict would be detected with greater intervals between transects (200m, 300m, 400m and 500m) for each forest. This was undertaken for all adults and males only. The relationship between transect interval and number of individuals detected reported higher correlation co-efficient when described by an exponential (R^2 = 0.947) rather than linear regression (R^2 = 0.813). Therefore, exponential relationships are described below.

The study areas also fell within the primary stratum (2km buffer around active lek sites) of the national winter survey being undertaken in 2015/16. Observations were made between November 2015 and March 2016 along line transects 2km in total length which took the form of an equilateral triangle (sides of 667m) (see Ewing *et al.,* 2012 for method details). The mean estimated strip width from the 2003/4 surveys was used (as detailed above) to calculate that 1.09km^2 (95% CI 0.95 – 1.26km^2) and 0.97 km^2 (95% CI 0.84 – 1.12km^2) was

surveyed in Kinveachy and Rothiemurchus respectively. Data from winter survey transects were considered if more than 50% of the transect fell within the study area boundary (nine transects in Kinveachy and eight transects in Rothiemurchus).

Faecal material was also collected during surveys (lekking = 58 samples, spring transects = 51 samples, brood counts = 12 samples). The initial project aim was to focus the genetic analyses on these samples, particularly those from leks, rather than analyse feather samples. Faecal samples were stored in plastic pots with silica gel but excessive mould and bacterial growth persisted on the sample surface due to a high moisture content and prevented extraction of viable capercaillie DNA. All genetic analysis was therefore based on DNA derived from feather material only.

2.2 Laboratory work

2.2.1 DNA extraction

A glossary of terms relating to the genetic analyses is provided in Annex 1.

To extract the DNA from feathers the manufacturers protocol for the DNeasy® Blood and Tissue kit (Qiagen), modified by the addition of 10 µL DTT during tissue digestion phase was used. Extracted DNA was quantified using a Nanodrop Spectrophotometer (ThermoScientific).

Where more than one feather was available within a sample, multiple extractions were performed simultaneously and the DNA later concentrated using Amicon Ultra filters (Merck Millipore) for downstream usage.

Overall 113 feathers were available for analysis, with sufficient viable DNA subsequently extracted from 106 samples. Feathers were not deemed suitable for DNA extraction when there was no identifiable calamus / quill (e.g. a single, small down feather).

2.2.2 Species identification

To check that a sufficient portion of the DNA extracted was of capercaillie origin rather than possible contaminants (e.g. bacteria, human *etc*.), polymerase chain reaction (PCR) was used to amplify and sequence a small fragment of the mitochondrial DNA (mtDNA). As mtDNA is inherited only from mother to offspring it gives a broad-scale evaluation of species and population diversity. Although it can be a powerful tool for confirming the species/subspecies status of animals and investigating taxonomy, its effectiveness is limited in fine scale population analysis as it provides no information on male ancestors. A 500 base-pair region of the Cytochrome Oxidase subunit I (CO1), a commonly used barcoding gene was PCR amplified and sequenced for a subset of samples (n=25), to confirm species identity. The quality of the mitochondrial sequences was checked by eye and aligned using Mega version 4 (Tamura *et al.,* 2007). The amplicon sequence was then compared to the online NCBI GeneBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using a BLAST search to check that the extracted DNA was from the expected species; all except two returned a positive match to capercaillie when compared against the NCBI GeneBank database. The two non-capercaillie samples were identified as belonging to an owl and a pigeon. These samples also failed to amplify using the microsatellite primers.

2.2.3 Individual identification

Nuclear DNA is inherited from both parents on chromosomes that reside in the cell nucleus. It often provides higher resolution than mitochondrial DNA for estimations of population diversity and relatedness. As it is inherited from both the mother and the father, it is also the only source of DNA suitable for assessing parent-offspring relationships, conducting individual identification and identifying the sex of the individual.

Microsatellites loci are one of the main genetic tools, or ‘markers’ used for examining inheritance in the nuclear genome. A literature search of recent publications (published from year 2000 onwards) revealed a suite of microsatellite markers capable of identifying individual capercaillie. Markers were derived from multiple studies (Piertney & Höglund, 2001; Segelbacher *et al.,* 2000) and had been optimised for use with faecal samples (Perez *et al.,* 2012). Fluorescently labelled primers were ordered from a suite of the published loci and polymerase chain reaction (PCR) profiles were optimized in the laboratory for DNA derived from tissue, faecal and feather samples. Although the availability of published PCR primers, designed to amplify microsatellite loci in capercaillie, eliminates the time consuming need to design new species-specific primers, they may require considerable optimisation

when transferred between laboratories or even when used on a 'new' population. Loci that failed to produce unambiguous genotypes were excluded resulting in a panel of nine markers that appeared sufficiently variable for use in identifying individual capercaillie within the sample set. Whilst it would be preferable to have included a higher number of markers to allow for some redundancy in the dataset, it was not possible to extend the panel size within the resources available for this study.

DNA extracts were PCR amplified using the optimized marker panels and genotyped on an ABI 3730 Sequencer (Applied Biosystems). Samples that failed to PCR amplify for less than 50% loci were re-run and only excluded if they failed to amplify repeatedly (minimum of three attempts). Samples that failed to amplify repeatedly at more than 50% of the loci were excluded. A sub-sample of individuals were genotyped repeatedly and used as positive controls to ensure consistency in the data produced. Details of the nine microsatellite loci that were used in this study are given in Table 2.

Allele scoring was conducted using GeneMapper v 4.0 (Applied Biosystems). Loci that failed to produce unambiguous genotypes for more than 90% of individuals were excluded from further analysis. Individual capercaillie that failed to produce clear genotypes for at least 70% of the remaining loci were also excluded from the final dataset.

Table 2. Details of primers used to genotype capercaillie individuals at nine microsatellite loci.

Locus name	Fluorescent label	Primer sequence (5'-3')	Allele size range	Author.
TUD4		FI:TCCCAAGGAGAGACAAAAGG	93-125	Pérez *et al*., 2012
TUD4	VIC	R:GGGAGGACTGTGTAGGAGAGC		Segelbacher *et al*., 2000
TUT3	6FAM	F:CAGGAGGCCTCAACTAATCACC	103-111	Segelbacher *et al.,* 2000
TUT3		RI:TGAGGGATTTATGCATGCTGC		Pérez *et al.,* 2012
TUD3	PET	F:TCCAAGGGGAAAATATGTGTG	120-122	Segelbacher *et al.,* 2000
TUD3		RI:TGGCTCTATTAAGGCTGCTG		Pérez *et al.,* 2012
TUT1		FI:GTATATCTGTCTGTCTGCCC	147-171	Pérez *et al*., 2012
TUT1	VIC	R:ATATGGCATCCCAGCTATGG		Segelbacher *et al.,* 2000
TUD1	6FAM	F:ATTTGCCAGGAAACTTGCTC	165-173	Segelbacher *et al*., 2000
TUD1		RI:TGAACATAACCTTTGCCTCC		Pérez *et al*., 2012
TUD7	VIC	F:TGACACTGGGGTCATTAGGC	157-161	Segelbacher *et al*., 2000
TUD7		RI:CCTCTGGTGAAAGGGAATGG		Pérez *et al.,* 2012
BG15	6FAM	F:AAATATGTTTGCTAGGGCTTAC	110-126	Piertney & Höglund, 2001
BG15		RI:GCTCCCTGTAAGGTAAATAGA		Pérez *et al*., 2012
BG18		FI:CAGAATTAATGTTTTGCTCTAAC	153-169	Pérez *et al.,* 2012
BG18	6FAM	R:CTGATACAAAGATGCCTACAA		Piertney & Höglund, 2001
TUD6		FI:GCCTTTTACTGCACTACTTGC	151-155	Pérez *et al.,* 2012
TUD6	PET	R:GAGGACTGCAGAACCCACTG		Segelbacher *et al.,* 2000

Data quality at each locus and population analysis were conducted using Genepop (www.genepop.curtin.edu.au; Raymond & Rousset, 1995), GenAlex (Peakall & Smouse, 2006), and the Adegenet package implemented in the R programming language (R Core Team, 2014). All microsatellite loci were assessed for the likely presence of null alleles (failure to amplify caused by mutations at the primer binding site) and allelic dropout (failure of both alleles to amplify) using the Microchecker software (Van Oosterhout *et al*., 2004) as this would affect the overall individual profiles using data from the sample replicates. No loci showed any sign of ambiguity across the duplicated control samples and large allelic dropout was not detected by the Microchecker software. The presence of null alleles was postulated for three loci (see Table 3) but is likely attributed to a higher amount of missing data for two out of three of these loci.

Although the samples were collected from two different forests (at least 6km apart), the similarity in allele frequencies (see Figure 2) meant that populations from each forest were not genetically distinguishable from each other using the nine microsatellite markers. Sampled areas were indistinguishable using Principle Components Analysis of genetic distances, where the samples from each forest overlapped (Figure 3) and no individual could be confidently assigned to a single 'population' using STRUCTURE (Pritchard *et al.,* 2000; data not shown). The samples from each study area were therefore considered as a single population before individual identification was undertaken.

Probability of identify (PI) was calculated using GenAlEx and used to estimate the minimum number of loci required to reliably identify and individual capercaillie. Multilocus genotypes were then assessed, taking into account potential null alleles to estimate the number of unique capercaillie individuals present in the current dataset.

Genotype profiles were repeatedly analysed using an increasing number of the microsatellite markers, to assess PI and the more conservative estimate PISibs (probability of identity estimates which allow for the presence of close relatives in the dataset). Within the samples analysed a minimum of five loci were needed to ensure that individual genotype profiles were unique and therefore identical profiles would signify a duplicated individual. However, five loci would not be able to confidently discriminate between full siblings with the high level of confidence usually sought for individual ID; the two estimators differ approximately two-fold in the number of loci required to achieve a PI (or PISibs) of <0.0001. Although 10 loci would be required to achieve a PI of < 0.0001, there continued to be only a 0.1 % (PI = 0.001) chance of two different individuals sharing a multi-locus genotype when nine loci were considered instead.

This suggests that even if closely related individuals are present in the dataset the current microsatellites can provide a confident, if conservative estimate of the number of individuals sampled (Figure 4). However, data are required at all loci (i.e. no missing data) for a 'match' to be considered significant, as missing data would further reduce the number of loci from which a conclusion was drawn.

Gimlet software (Valière, 2002) was used to compare each individual genotype profile to the rest of the dataset, to calculate the number of unique genotypes in the sample set, identify individuals with matching multilocus genotypes, and in turn provide a lower limit estimate of the number of individuals represented.

In addition to calculating the total number of unique multilocus genotypes which represents the minimum number of individuals present in the sample set, capture-mark-recapture (CMR) estimates specifically developed for DNA-based recaptures were attempted using CAPWIRE software (Palsbøll, 1999; Miller *et al.,* 2005).

Table 3. Summary statistics for each of the nine loci genotyped. N = number of individuals genotyped, Na = number of alleles detected, Ho = Observed heterozygosity, He = Expected heterozygosity. The proportion of missing data for each locus is given and loci where null alleles are highlighted.

Locus name	N	Na	Ho	He	Missing data	Null alleles suspected
TUD4	82	6	0.683	0.651	0.06	no
TUT3	86	6	0.721	0.722	0.01	no
TUD3	86	3	0.395	0.533	0.01	yes
TUT1	68	5	0.485	0.716	0.21	yes
TUD1	80	5	0.663	0.694	0.08	no
TUD7	77	3	0.571	0.594	0.12	no
BG15	68	3	0.162	0.599	0.21	yes
BG18	83	4	0.602	0.622	0.05	no
TUD6	85	6	0.671	0.752	0.02	no
Average	79.4	4.5	0.550	0.654	0.09	

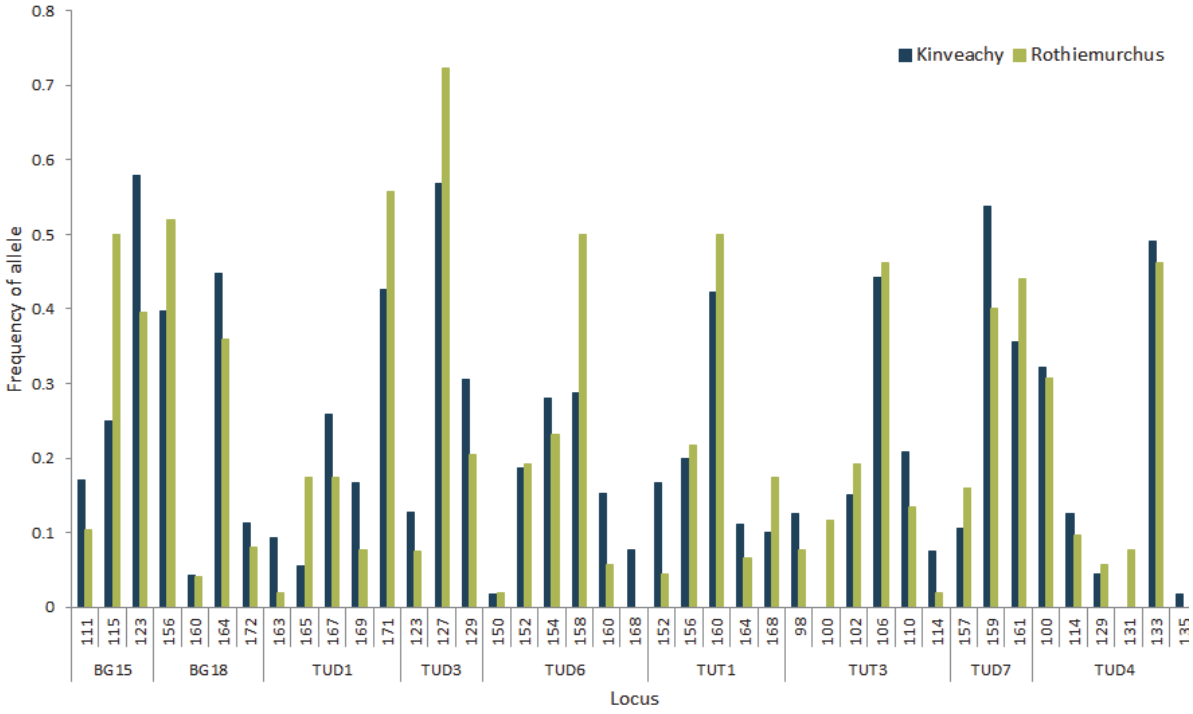

Figure 2. The frequency of each allele at a given locus resolved for each of the Kinveachy and Rothiemurchus sample sites. With the exception of a few alleles, the frequencies of occurrence for each allele are similar between the putative populations.

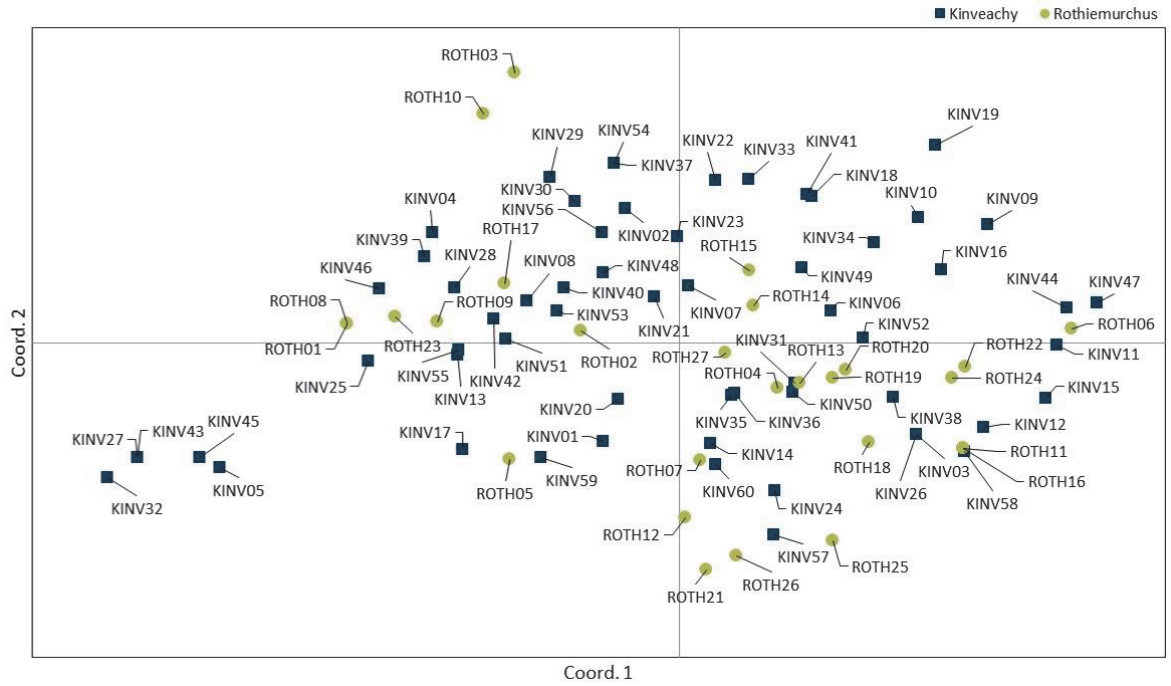

Figure 3. Principle Components Analysis of genetic distance for all samples. Each point represents an individual bird and their proximity in the plot space represents their genetic similarity. The lack of any obvious segregation between samples from the different collection sites suggests that individuals are derived either from a single population or two recently derived ones.

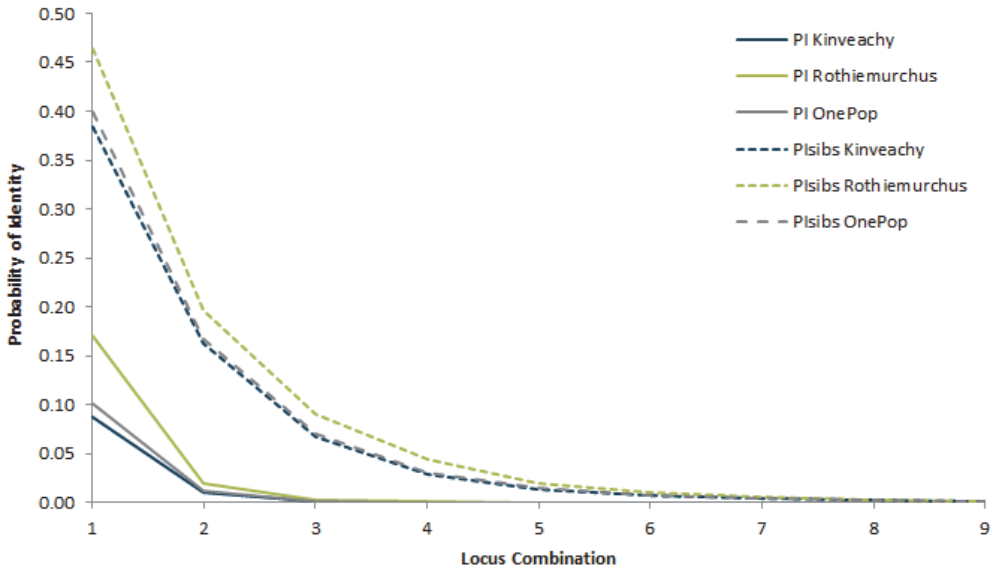

Figure 4. Probability of Identity (PI) and the more conservative PISibs were estimated by calculations of the proportion of individuals that share an identical genotype. All estimates were performed for both populations independently and by considering them as one population (OnePop). Abbreviations used for the data plotted are as follows: PI = Average probability that two independent samples will have the same identical genotype. PIsibs = Average probability of identify when related individuals are included in the sample set.

2.2.4 Molecular sexing

Within nuclear DNA a portion of the intron-containing CHD-1 gene that is located on both sex chromosomes, was amplified using primers published by Jensen *et al.,* (2003). Contrary to what is observed in mammals, female birds are the heterogametic sex, i.e. have two different sex chromosomes (ZW) in bird species whereas males are homogametic (ZZ). When the PCR products are examined on an agarose gel, the sex of each sample can be calculated by the presence of either a single (male) or double (female) bands (Solari, 1994). Details and thermal profile conditions for molecular sexing are provided in Jensen *et al.,* (2003). Sex determined by molecular techniques was compared to assessments made in the field. Where discrepancies were observed, molecular sexing was repeated up to three times to ensure consistency of results.

G-tests were used to compare the sex-ratio from feather samples between forests and with an equal sex-ratio.

3. RESULTS

3.1 The number of individual capercaillie from DNA analysis

Repeated failure to amplify or produce unambiguous genotypes across multiple loci was due to the presence of either low DNA quality or a non-focal species sample (i.e. not capercaillie). Ambiguous genotypes were scored as missing data and of the 106 samples that yielded DNA, 19 were excluded from full analysis as they failed to amplify at more than six microsatellite loci. The final dataset included 87 capercaillie samples (60 Kinveachy and 27 Rothiemurchus) genotyped at between seven and nine microsatellite loci. Details of all feather samples used can be found in Annex 2.

Overall, approximately 80 individual genotype profiles were detected by the Gimlet software (55 in Kinveachy and 25 in Rothiemurchus). The estimated minimum number was 69-82 individuals, taking into account potential allelic dropout (see Table 3) which would cause a misidentification of duplicate pairs as detailed below.

Seven pairs of matching multi-locus genotypes were found among the 87 individual samples tested. Five pairs of individuals demonstrated the same genotype at all nine loci, and therefore were considered duplicate samples (i.e. derived from the same individual). Two pairs of individuals were missing data for one or two loci and so could not be assumed to be a true match. If these individuals do have the same alleles at the missing loci, then they would represent further duplicate samples in the dataset and so the minimum estimate of 80 individuals would hold true. Furthermore, among the sample pairs that demonstrated differences at either one or two loci, approximately 47.4% may be attributed to allelic dropout. Owing to three-way matches among individuals, the number of pairwise matches is greater than the number of individuals involved and overall estimates of unique individuals in the dataset are calculated to take this into account using GenAlex software.

The seven duplicated genotypes among the 87 samples tested equates to a recapture rate of 1.09. All recaptured individuals remained in the same forest with a mean ± s.e. = 1.71 ± 0.73km distance between sample locations (Table 4).

Table 4. Details of genotyped capercaillie identified as the same individuals from nine matching microsatellite loci (missing data at one locus).*

Forest	Matched Individual DNA Analysis IDs		Sample source	Molecular sexing	Distance between locations (km)
Kinveachy	KINV03	KINV26	brood count	M	-
Kinveachy	KINV29*	KINV30	brood count	M	0.86
Kinveachy	KINV27	KINV43	brood count	M	0.55
Kinveachy	KINV05*	KINV45	brood count	M	2.52
Kinveachy	KINV37	KINV60	brood count	M	5.02
Rothiemurchus	ROTH01	ROTH08	lek / brood count	M	0.53
Rothiemurchus	ROTH11	ROTH16	spring transect / brood count	M	0.75

Seventy-seven samples were successfully sexed by molecular technique, of which 72 were also individually genotyped (plus five which were not individually genotyped). This equates to 68% success from all feather samples and 83% success from those with high quality DNA which yielded individual genotypes (full details in Appendix 2). Of the 77 feather samples sexed using molecular methods, 40 had field data available. Across these samples, mismatches between field and molecular data were observed for six samples (15%), four were identified as male by molecular sexing and female in the field and the other two *vice versa*.

Sexing the samples using DNA the sex ratio was 3.2: 1 male : female in Kinveachy and 1.6: 1 male : female in Rothiemurchus, however the forests did not differ significantly (G_{adj} = 1.78, P = 0.182). The skew towards males was significantly different from 1: 1 in Kinveachy, but not in Rothiemurchus (Table 5).

Table 5. A comparison of the identified sex for capercaillie sample using molecular sexing. Five duplicates males removed from Kinveachy and two from Rothiemurchus when calculating the number of each sex. G-tests compared the number of males and females with 1:1 ratio.

	Male	Female	Sex ratio	G_{adj}	P
Using molecular sexing only:					
Kinveachy	39	12	3.2	8.24	0.004
Rothiemurchus	16	10	1.6	0.68	0.408

3.2 The number of individual capercaillie detected in relation to survey effort

The brood count surveys yielded the majority of the feather samples (73%) and the majority of the individuals detected (67 individuals; 84% of the 80 individuals across both forests). By estimating the distance surveyed during brood counts (transects 100m apart) we can estimate how many individuals we would expect to detect at wider transect intervals (e.g. if transects 200m, 300m, 400m or 500m apart were surveyed; Figure 5) and therefore how many individuals may have been detected if all the ground had been surveyed (x = 0 within Figure 5).

The number of individuals detected from genetic analysis, when collecting samples at 100m intervals, was lower than the number reported from observation during brood counts (15% lower in Kinveachy and 43% lower in Rothiemurchus) and was higher than the number of lekking males in Kinveachy (7%) but lower than lekking males in Rothiemurchus (71%).

Within Kinveachy if more samples were collected the maximum number of individuals detected is estimated to be similar or higher than recorded during brood counts or lek surveys, but still remains lower than these field methods in Rothiemurchus (Figure 5).

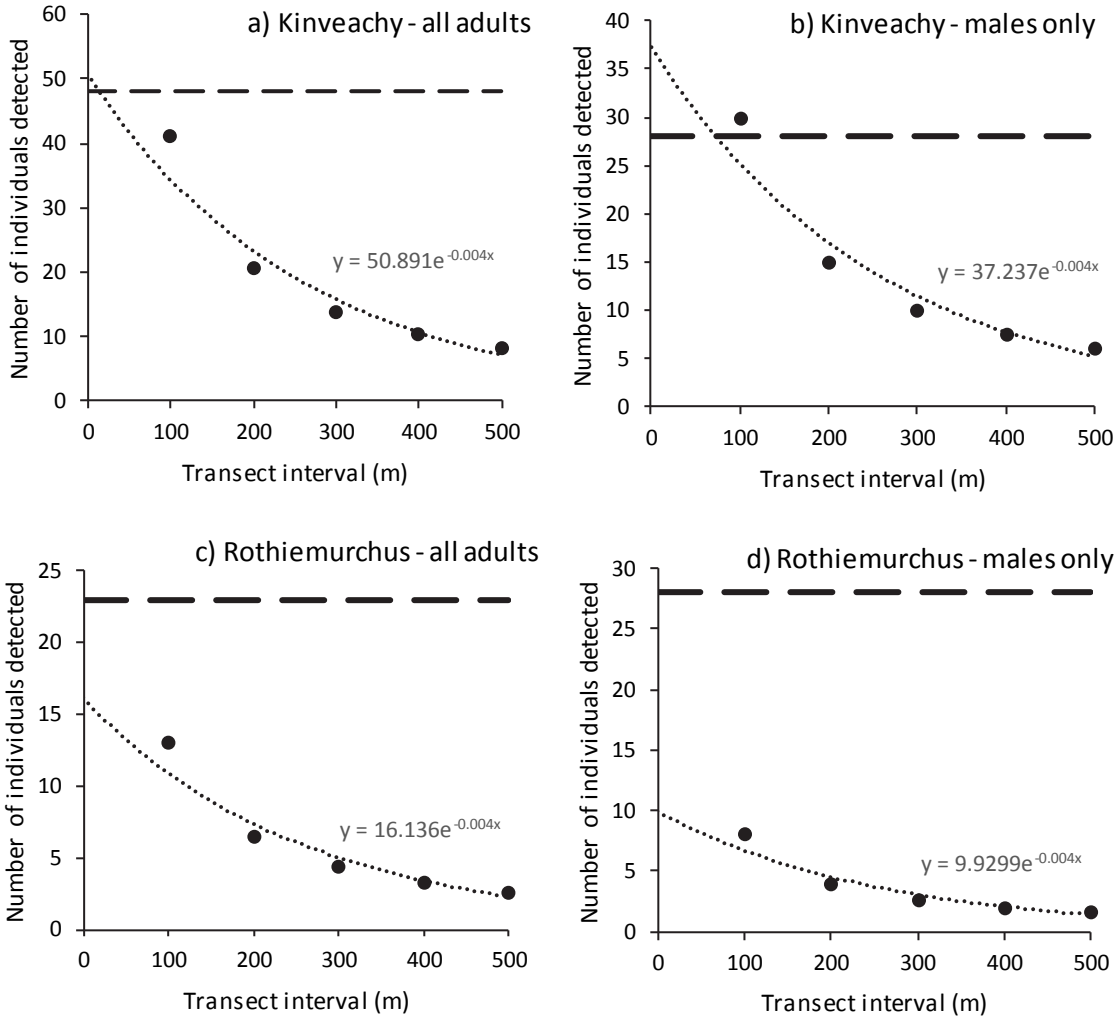

Figure 5. Using the location of each individual identified from genetic analysis of feather samples collected during brood counts (100 m interval between transects), the number of individuals that would have been detected at wider transect intervals has been calculated for Kinveachy and Rothiemurchus, for both all adults and males only. Dashed line illustrates the total number of adults reported during brood counts (all adults) or lek counts (males only). Dotted trend line and equation illustrate the exponential relationship between the transect interval and the number of individuals.

This comparison suggests that doubling of effort in Kinveachy (equivalent to transects at 50m intervals) may yield 42 individuals (6% less than recorded on brood count) and 30 males (9% higher than recorded on lek surveys). In Rothiemurchus a doubling of sampling effort may yield 13 individuals (as found at 100m transect interval) and eight males (71% lower than recorded on lek surveys).

3.3 Comparison of genetic analysis with other field data

The estimated population size from genetic analysis (excluding seven recaptures: 80 individuals, across both forests; 55 in Kinveachy and 25 in Rothiemurchus) has been compared with other survey techniques (Table 6). Calculation of confidence limits was not possible due to the low recapture rate.

Twenty-eight lekking males were recorded within both study plots in 2014, however the number of males and females combined was greater on Kinveachy (48 birds, compared to 36 in Rothiemurchus). The number of males and all adults recorded at leks was higher than the number detected from genetic analysis on Rothiemurchus, but lower on Kinveachy (Table 6).

The population estimates within the study area were three to four times higher from spring transects (Kinveachy 154 (95%CL 133-178) and Rothiemurchus 99 (95% CL 86-115)) and winter survey (Kinveachy 171 (95%CL 148-198) and Rothiemurchus 100 (95% CL 86-115)) than recorded from genetic analysis for both forests.

The number of individuals from brood count surveys were more similar to those reported from the genetic survey but 13% lower with 48 individuals in Kinveachy and 8% lower with 23 in Rothiemurchus.

When considering each sex it is clear that lek surveys, spring transects and genetic analyses recorded fewer females relative to brood counts, whereas brood counts recorded fewer males relative to lek survey, spring transects and genetic analyses (Table 6).

Table 6. Number of individual capercaillie recorded during field surveys and genetic analysis in two Scottish forests. Where distance sampling techniques have been used the 95% CI have also been included in parentheses.

	Survey method	Kinveachy	Rothiemurchus
All adults:	Lek survey	48	36
	Spring transects	154 (133 - 178)	99 (86 - 115)
	Brood count survey	48	23
	Winter survey	171 (148 - 198)	100 (86 - 115)
	Genetic survey	55	25
Males:	Lek survey	28	28
	Spring transects	104 (102 - 136)	84 (83 - 110)
	Brood count survey	18	7
	Winter survey	n/a	n/a
	Genetic survey	33	14
Females:	Lek survey	20	8
	Spring transects	28 (27 - 37)	15 (15 - 19)
	Brood count survey	30	16
	Winter survey	n/a	n/a
	Genetic survey	11	8

4. DISCUSSION

The success of utilising feather samples from which to extract DNA is well documented in the literature (Pearce *et al*., 1997; Segelbacher, 2002; Horvath *et al*., 2005; Hogan *et al.,* 2008). Provided the feather is of suitable size and quality it provides a reliable source from which to extract DNA suitable for microsatellite genotyping. In the present study 82% of feathers were large enough to be processed for DNA extraction and successfully genotyped as individuals at seven or more loci, compared to 50% success (Segelbacher, 2002) and 100% success (Segelbacher & Piertney, 2007) reported in previous studies. The type of feather has been found to be less important than the condition of the feather, so only the freshest feathers should be analysed (Hogan *et al.*, 2008). However, we found very small down-like feathers tend to provide a less reliable source for collecting a suitable yield of DNA to meet the needs of microsatellite genotyping. Collecting only fresh feathers is also likely to reduce the likelihood of feathers from the previous year being analysed.

The remainder of the discussion has been ordered with respect to the objectives of this project followed by a section with recommendations for future work.

4.1 To evaluate the intensity of genetic sampling required to estimate population size

A key component of any genetic analysis is the sampling strategy. The majority of samples collected within this pilot were from transects walked during brood counts approximately 100m apart. Where individuals move around in groups an adaptive cluster sampling method can be applied (Thompson 1991; Ebert *et al.* 2012), however this method would not be applicable to adult capercaillie. Other studies of capercaillie have generally described the search effort as systematic rather than specifying the interval between transects (except transects 10m apart walked in Rösner *et al.*, 2014). This pilot study found five to ten samples (depending on the number of loci being considered) thought to be from the same individual (6 – 11% of the 80 individuals genotyped, within 2070 ha study area). This is much lower than other studies where 40% of the 56 individuals were found more than once when samples were collected during a single visit to leks within 50,000 ha study area (Morán-Luis *et al.,* 2014) and 86% of 556 individuals were found more than once when samples from two visits were collected over 14 months with in 120,000 ha study area (Rösner *et al*., 2014). However, in each of these cases a much larger number of samples were collected; almost 300 and 7,500 (of which 550 were analysed) respectively.

The number of feathers collected during the brood counts was 1 to 1.25 times the number of birds recorded during the same surveys. It has been suggested that 2.5 observations per individual be recorded to obtain population estimates within 15% range of the true population size (Miller *et al.,* 2005). For the number of samples to be 2.5 times, the number of birds surveys to collect samples would need to be repeated (double the survey effort). An alternative way to look at survey effort is to examine the number of individuals identified by the genetic analyses. By comparing transects at intervals from 100 – 500m undertaken in August (Figure 5) we found that for Kinveachy by increasing the effort we would expect to identify the same number (or more) individuals than detected in field surveys, but for Rothiemurchus the genetic analyses may not identify as many individuals as field methods. This difference between sites could be due to lower density of birds in Rothiemurchus. Therefore, we would recommend that for at least one visit the focus is solely on sample collection with the aim of increasing the number of samples per distance surveyed.

Achieving more “recaptures” would provide a more robust population estimate from CMR models. Consistent sampling effort across all study areas will be important. Within this pilot all feathers collected were analysed, however feathers found within 1m of each other were assumed to be from the same individual and stored as such. Where multiple feathers were

labelled as belonging to the same individual, only a single feather was subjected to genetic analysis. More intensive studies recommend that samples within 25 - 30m (Morán-Luis *et al.,* 2014; Rösner *et al.*, 2014; Mollet *et al*., 2015) or 100m (Jacob *et al*., 2010) are disregarded to reduce repeat sampling of the same individual.

That fewer feathers were found in April-May compared to August may result from both greater feather availability post-moult and greater survey intensity in the latter period. In April-May, 18 out of 20 individuals identified differed from those identified from the August samples. Mortality of birds could contribute to this difference, however if these individuals had died it would equate to 22% mortality over three months, which is higher than previously recorded (8% per three months in Moss *et al.,* 2000). Therefore, we suggest that either birds had moved from within the study area and / or not all birds were detected from the August feather collection confirming that higher sampling intensity is required.

Females delay moult if they have chicks (Watson & Moss, 2008) hence collecting feathers post-breeding in September / October, particularly in good breeding years, may improve female detection. An initial feather collection combined with brood surveys could be supplemented with later survey with the sole purpose of collecting samples.

Another way to increase the number of samples would be to collect both feathers and faecal material. Whilst not achieved in this study, extraction of DNA from faecal samples is feasible if samples are collected and stored appropriately. Previous studies have reported success rates of 48% when extracting and amplifying DNA from faecal material for eight or more microsatellite loci (Jacob *et al.,* 2010), 60% from faecal material for seven or more microsatellite loci (Morán-Luis *et al.,* 2014), 88% from faecal material for nine loci (Mollet *et al.,* 2015) and 99% of a sub sample of faecal material using ten microsatellite loci (Rösner *et al*., 2014). These were achieved in studies that used two to three day-old faecal samples deposited on snow and frozen on the day of collection (Rösner *et al.,* 2014; Mollet *et al*., 2015); fresh (wet) samples collected during summer and frozen on the same day (Rösner *et al.*, 2014); or air-dried or stored with a silica:sample ratio of 1:3 and frozen (Jacob *et al.,* 2010). Moisture and ultra-violet light degrade and destroy DNA, with the age of samples decreasing amplification success, genotyping rates and increasing the allelic dropout and false allele rates (Rehnus & Bollmann, 2016, and references within).

4.2 To determine the relationship between local forest population size estimates derived from genetic analysis, to those from cocks attending leks in spring and adults seen during summer brood counts

Unlike standard CMR studies, in DNA-based approaches an individual can be captured more than once per session (i.e. can be detected in more than one sample). Methods such as CAPWIRE can estimate abundance from a single sampling session as long as individuals are sampled sufficiently to estimate recapture probabilities and assuming that genotype profiles can unambiguously distinguish between individuals (Miller *et al.,* 2005, Puechmaille & Petit, 2007). Due to the low recapture rate within this study this software did not generate population estimates therefore a minimum number of individuals could only be based on the number of unique multilocus genotypes present within the current sample set.

Within the genetic analysis genotyping errors may lead to an overestimation of abundance because they increase the minimum population size detected and lower the probability of recapture (McKelvey & Schwartz, 2004; Lukacs & Burnham, 2005). Alternatively, a ‘shadow effect’ can occur when individuals share the same molecular ‘tag’ (i.e. multilocus genotype) due to using an insufficient number of variable molecular markers (Mills *et al.,* 2000). Allelic drop out or the presence of null alleles can also confound genotype profiles. When the shadow effect is present, individuals cannot be distinguished from each other, leading to underestimation in population size. The effect of genotyping error on population estimations

can be minimised by using a 'matching approach (Creel *et al*., 2003). Rather than consider the level of mismatch across different loci, probability of identity (PI) and probability of identity of siblings (PI_{SIB}) can be used to assess the amount of matching alleles between two individual samples and the probability that the matches occur by chance from two different individuals. The PI and PI_{SIB} estimates reported here suggest that the current marker panel should reliably distinguish individual capercaillie so long as data are present for all loci, but that confidence is reduced when close relatives (i.e. full siblings) are included in the dataset. Although increasing the number of markers used could be expected to increase the power of the panel, a 9-marker panel has previously been used to successfully identify individual capercaillie in the north west of Spain (Morán-Luis *et al*., 2014), suggesting that this is a reasonable number of loci to use.

Most studies which apply genetic CMR analyses produced population size estimates 30–50% larger than estimates obtained with field methods (e.g., Solberg *et al*., 2006; Zhan *et al*., 2006; Guschanski *et al*., 2009; Kendall *et al*., 2009; Cubaynes *et al*., 2010). In addition to the lek and brood survey data, spring and winter transect survey data were collated. The genetics analysis within this pilot study found fewer individuals than estimated from spring and winter transects (after correcting for area covered using estimated strip widths). The number of birds within each forest was approximately three times greater for the surveys using distance sampling than for other methods. The spring transect surveys could provide inflated estimates, particularly of males, if the transect lines passed through leks. Across both forests, four out of the eight leks were crossed by a transect line. However, this does not explain the high population estimates from winter surveys, when males are expected to be less clustered.

The brood and lek surveys produced estimates closer to those from the genetic analysis. The brood surveys reported 8-13% fewer individuals than the genetic analysis, however the lek survey reported higher numbers in one forest, but lower numbers in the second forest, when compared to the genetic analysis. This suggests that within the pilot study not all individuals were sampled for genetic analysis.

The genetic analysis also had fewer females than reported during the brood survey. Double counting is minimised whilst undertaking brood surveys by using multiple observers, but even if a low level of double counting has occurred, the number of females detected by genetic analysis still appears much lower than expected. This may be due to the non-amplification of one allele during molecular sexing resulting in female individuals being sexed as male. Repeated genotyping is required for all samples that initially appear homogametic (female) to ensure the absence of a second allele is genuine. However even in studies where all suitable habitat was searched, differences in detection probabilities between sexes have been reported (Mollet *et al*., 2015). Mollet *et al*. hypothesised that females are probably moving less conspicuously and looking for cover against predators more than males, because they are exposed to more potential predator species due to their smaller body size. Hence, female droppings and feathers may be less conspicuous than males', which would lead to them being undetected with the sampling intensity applied in this study. Future work should, therefore, include effort to increase the samples particularly from females within the population, by undertaking surveys with the sole purpose of collecting samples in September / October.

4.3 Using results from objectives A and B, assess whether genetic techniques can be a cost effective and accurate means of determining Scottish population size

It has been suggested that 2.5 observations per individual be recorded to obtain population estimates within 15% range of the true population size (Miller *et al.,* 2005), however lower levels of recapture are reported in the majority of published studies. Solberg *et al.,* (2006) also recommended that studies using non-invasive genetic methods based on faecal

samples should aim at collecting 2.5 to 3 times the number of faecal samples as the "assumed" number of animals (considering that in their laboratory analysis approximately 20 – 30% of the samples could not be genotyped). Using the 2009/10 winter survey population estimate of 1,285 individual capercaillie in Scotland this would suggest that approximately 3,200 – 3,900 samples need to be collected over approximately 82,000ha (or 2,400 - 2,900 samples in Badenoch & Strathspey which holds 75% of the population). This would require sampling intensity of 0.04 – 0.05 samples per ha which is similar to that achieved within this pilot study (0.05 samples per ha). However, we know this intensity of sampling yielded low recapture rates and therefore more samples need to be collected and sufficient funds allocated to permit samples to be repeatedly genotyped, as is standard with non-invasive sample genotyping (Taberlet *et al.,* (1996), but also see Morán-Luis *et al.,* 2014; Rösner *et al.,* 2014; Segelbacher 2002). This 'multi-tube' approach would be expected to reduce the amount of error present within the genotype data and together with more structured sampling, could yield a reliable estimate.

A criticism of using distance sampling for the national survey is the fact that it is difficult to detect trends in the numbers of birds due to the large confidence intervals around the population estimates. Considering the four national surveys in Scotland the 95% confidence limits were on average ± 39% of the mean in the most recent published survey (Ewing *et al.*, 2012), but were greater in the 1998/9 survey when the upper confidence limit was almost double the mean (Wilkinson *et al*., 2002). Using genetic analysis where the average recapture rate was 2.4 estimated the greatest 95% confidence limits to be 2 – 11% of the mean total population estimate using eight population size models (556 individuals, 95% CL 544 – 616, Rösner *et al.,* 2014). The confidence limits were wider when smaller regions were considered (Šumava National Park 234 (95%CL 216-323) and Bavarian Forest National Park 154 (95% CL 141-218). Where lower average recapture rates were reported (1.9 ± 1.1 for males and 1.7 ± 1.2 for females) and the population estimate ranged from five to 78 individuals, the average 95% confidence limit was 38% of the mean (Jacob *et al*., 2010). This suggests that if a sufficient recapture rate is achieved, using multiple population size estimators with genotype data are likely to provide more robust population estimates than possible with distance sampling. However, detecting regional / local trends where the population estimates are lower may be hampered by wider confidence intervals.

The primary stratum of the national survey (2km buffer around leks) is approximately 82,000ha. To undertake transects 100m apart, approximately 8,200km of transect would need to be surveyed. Undertaking these transects twice to increase the recapture rate would mean surveying 16,400km. Assuming 10km a day can be surveyed then this would equate to 1,640 man-days. Therefore, it may be more realistic to focus on greater sampling effort within a smaller geographical area and / or combine at least a proportion of the search for samples with other field surveys being undertaken in the forests. Further pilot work, including sites with a range of densities, would be recommended to confirm our estimate for the level of sampling needed.

From this pilot study we suggest that at least a doubling of effort would be required to collect enough samples to calculate a robust population size. This does not seem cost-effective at a national scale unless sufficient resources are available. With limited resources it may be more useful to monitor populations at a smaller geographical scale, with greater sampling intensity.

4.4 Recommendations

1. ***Sample collection.*** Sample storage should be carefully considered, with only fresh feather (and faecal material) collected and frozen within 12 hours. Samples should be sexed on size and colour in the field, with data compared to that gained from molecular sexing to enable a true comparison of both methods and estimate of the error rate for each. Feathers found separately (more than 0.5m apart) should be assumed to be from separate individuals until proven otherwise. Accurate location information for all samples should be collected so that samples in close proximity may be sub-sampled.

2. ***Sampling intensity for whole forest population monitoring.*** This is expected to need to be at least twice as intensive as undertaking during this pilot to increase the number of samples and therefore the recapture rate. Samples could be collected during brood counts and then same areas revisited with additional surveys in September-October to increase the sampling intensity. For forests where brood counts are not routinely undertaken, two visits by staff / volunteers may be necessary. This was suggested for monitoring populations of the Bohemian forest population in Germany, where a five-year census was considered sufficient for estimation of population size and movements (Rösner *et al.,* 2014). Further pilot work would be recommended to confirm the level of sampling required, particularly for sites with lower density of birds.

3. ***Genetic analysis.*** If genetic tools were to be included in routine monitoring of capercaillie populations, attempts should be made to improve the sampling strategy employed and identify potential improvements to the marker system used, either by investigating more published microsatellite markers or by the identification of Single Nucleotide Polymorphism (SNP) markers. The latter would involve initial investment of good quality samples (tissue and blood from fresh carcasses) from which to identify informative loci, development of laboratory assays that could be applied in a more economical fashion downstream, and census techniques based on a pedigree reconstruction from SNP data. The benefits of these markers are multiple and include: the ability to initially identify 100's-1000's loci simultaneously, improved ease of transfer between laboratories and comparison of datasets, and production of sequence data that can provide added legacy for the project; re-analysis of which might be useful in the future for functional genetic studies, were for example a whole genome of the species to become available.

4. ***Use of faecal samples.*** Analysing both feathers and faecal material would boost the number of samples. Freezing all samples (feather and faecal) within 12 hours of collection and minimise the number of freeze/thaw cycles experienced by samples would help maintain the quality of the DNA. Alternatively employing a more recently developed swabbing technique to sample the surface of the faecal sample (rather than attempt to preserve the entire sample) has proven an effective method of collecting DNA containing cells in other species (Rutledge *et al.,* 2009; Ramón-Laca *et al*., 2015) and is currently being trialled with capercaillie faecal samples at the RZSS WildGenes laboratory. Faecal samples are most abundant at leks and may be useful at improving the accuracy of the counts of males at leks.

5. CONCLUSIONS

This pilot study collecting samples of faecal material and feathers from two forests provided insights into the likely success of using genetic analyses for estimating population size of capercaillie in Scotland.

Collection of fresh feathers during the post-moult period would provide information on the numbers of both males and females. Considerable man-power may be required to collect samples from the area currently used for the national survey (estimated as 1,640 man-days), although this need not be undertaken annually. As with traditional CMR techniques, intensive searches would be needed to ensure the recapture rates were sufficient for population estimate models to calculate lower confidence intervals than have been possible in recent distance sampling surveys. However, estimates based on genetic data are beneficial as they can be applied to data collected during a single sampling session. Combining sample collection with other surveys in forests and / or restricting the area being monitored would also reduce the costs of sample collection.

The faecal material requires better storage than achieved within this pilot study. From other capercaillie studies 48% or more of the samples can yield individual genotypes. Faecal material is more often found at leks and searching lek areas could be used to improve the estimate of males. However, females are likely to be under represented as they spend less time at leks. Collecting samples from leks would require less man-power than surveying wider areas of suitable habitat, but would be less suitable for assessing the population size in regions where the lek location is unknown. Molecular sexing provides an efficient opportunity to identify individual sex from the same samples collected for population size estimates. By determining the sex of all samples the sex ratio could be determined, and if strongly biased towards males then the number of males alone could be used to estimate the population (assuming an equal sex ratio).

6. REFERENCES

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., Borchers, D.L. & Thomas, L. 2001. *Introduction to Distance Sampling: Estimating Abundance of Biological Populations.* Oxford University Press, Oxford.

Catt, D.C., Baines, D., Picozzi, N., Moss, R. & Summers, R.W. 1998. Abundance and distribution of capercaillie *Tetrao urogallus* in Scotland 1992-1994. *Biological Conservation,* 85, 257-267.

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

Cubaynes, S., Pradel, R., Choquet, R., Duchamp, C., Gaillard, J., Lebreton, J., Marboutin, E., Miquel, C., Reboulet, A., Poillot, C., Taberlet, P. & Gimenez, O. 2010. Importance of accounting for detection heterogeneity when estimating abundance: the case of French Wolves. *Conservation Biology,* 24, 621–626.

Eaton, M.A., Marshall, K.B. & Gregory, R.D. 2007. Status of Capercaillie *Tetrao urogallus* in Scotland during winter 2003/04. *Bird Study,* 54, 145–153.

Ebert, C. Knauer, F., Spielberger, B., Thiele, B. & Hohmann, U. 2012. Estimating wild boar *Sus scrofa* population size using faecal DNA and capture-recapture modelling. *Wildlife Biology,* 18, 142-152.

Ewing, S.R., Eaton, M.A., Poole, T.F., Davies, M. & Haysom, S. 2012. The size of the Scottish population of Capercaillie *Tetrao urogallus*: results of the fourth national survey. *Bird Study*, 59, 126-138.

Guschanski, K., Vigilant, L., McNeilage, A., Gray, M., Kagoda, E. & Robbins, M. 2009. Counting elusive animals: comparing field and genetic census of the entire mountain gorilla population of Bwindi Impenetrable National Park, Uganda. *Biological Conservation,* 142, 290–300.

Hogan, F.E., Cooke, R., Burridge, C.P. & Norman, J.A. 2008. Optimizing the use of shed feathers for genetic analysis. *Molecular Ecology Resources*, 8, 561-567.

Horvath, M.B., Martinez-Cruz, B., Negro, J.J., Kalmar, L. & Godoy, J.A. 2005. An overlooked DNA source for non-invasive genetic analysis in birds. *Journal of Avian Biology,* 36, 84–88.

Jacob, G., Debrunner, R., Gugerli, F., Schmid, B. & Bollman, K. 2010. Field surveys of capercaillie *Tetrao urogallus* in the Swiss Alps underestimated local abundance of the species as revealed by genetic analyses of non-invasive samples. *Conservation Genetics,* 11, 33-44.

Jensen, T., Pernasetti, F.M., & Durrant, B. 2003. Conditions for rapid sex determination in 47 avian species by PCR of genomic DNA from blood, shell-membrane blood-vessels and feathers. *Zoo Biology*, 22, 561-571.

Kendall, K.C., Stetz, J.B., Boulanger, J., Macleod, A.C., Paetkau, D. & White, G.C. 2009. Demography and genetic structure of a recovering grizzly bear population. *Journal of Wildlife Management,* 73, 3–17.

Lukacs, P.M. & Burnham, K.P. 2005. Review of capture-recapture methods applicable to noninvasive genetic sampling. *Molecular Ecology,* 14, 3909–3919.

McKelvey, K.S. & Schwartz, M.K. 2004. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. *The Journal of Wildlife Management,* 68, 439-448.

Miller, C.R., Joyce, P., Waits, L.P. 2005. A new method for estimating the size of small populations from genetic mark-recapture data. *Molecular Ecology,* 14, 1991–2005.

Mills, L., Citta, J., Lair, K., Schwartz, M.K. & Tallmon, D. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecological Applications*, 10, 283–294.

Mollet, P., Kéry, M., Gardner, B., Pasinelli, G. & Royle, J.A. 2015. Estimating population size for capercaillie (*Tetrao urogallus* L.) with spatial capture-recapture models based on genotypes from one field sample. *PLoS ONE,* 10 (6), e0129020.

Morán-Luis, M., Fameli, A., Blanco-Fontao, B., Fernández-Gil, A., Rodríguez-Muñoz, R., Quevedo, M., Mirol, P., & Bañuelos, M.J. 2014. Demographic status and genetic tagging of endangered capercaillie in NW Spain. *PLoS ONE*, 9 (6), e99799.

Moss, R. 1994. Decline of the capercaillie (*Tetrao urogallus*) in Scotland. *Gibier Faune Sauvage*, 11, 217–222.

Moss, R., Picozzi, N., Summers, R.W. & Baines, D. 2000. Capercaillie *Tetrao urogallus* in Scotland – demography of a declining population. *Ibis,* 142, 259–267.

Palsbøll, P. 1999. Genetic tagging: contemporary molecular ecology. *Biological Journal of the Linnean Society,* 68, 3–22.

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

Pearce, J.M., Fields, R.L. & Scribner, K.T. 1997. Nest materials as a source of genetic data for avian ecological studies. *Journal of Field Ornithology,* 68, 471–481.

Perez, T., Fernando Vázquez, J., Quirós, F. & Domínguez, A. 2012. Erratum to: Improving non-invasive genotyping in capercaillie (*Tetrao urogallus*): redesigning sexing and microsatellite primers to increase efficiency of feces samples. *Conservation Genetic Resources*, 4, 821-823.

Piertney, S. 2004. *Genetic diversity of Scottish populations of Capercaillie* Tetrao urogallus*.* Final report prepared at completion of Phase II of contract 'Scottish Capercaillie Genetics Project'. Unpublished report.

Piertney, S.B. & Höglund, J. 2001. Primer note: Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). *Molecular Ecology Notes*, 1, 303-304.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of Population Structure Using Multi-locus Genotype Data. *Genetics*, 155, 945–959.

Puechmaille, S.J, & Petit, E.J. 2007. Empirical evaluation of non-invasive capture-mark-recapture estimation of population size based on a single sampling session. *Journal of Applied Ecology,* 44, 843–852.

Ramón-Laca, A., Soriano, L., Gleeson, D. & Godoy, J.A. 2015. A simple and effective method for obtaining mammal DNA from faeces. *Wildlife Biology,* 24, 195-203.

Raymond, M. & Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity,* 86, 248-249.

R Core Team 2014. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. URL http://www.R-project.org/.

Rehnus, M. & Bollmann, K. 2016. Non-invasive genetic population density estimation of mountain hares (*Lepus timidus*) in the Alps: systematic or opportunistic sampling? *European Journal of Wildlife Research*, 62, 737-747.

Rösner, S., Brandl, R., Segelbacher, G., Lorenc, T. & Müller, J. 2014. Non-invasive genetic sampling allows estimation of capercaillie numbers and population structure in the Bohemian Forest. *European Journal of Wildlife Research*, 60, 789-801.

Rodriguez-Muñoz, R., Mirol, P.M., Segelbacher, G., Fernández, A. & Tregenze, T. 2007. Genetic differentiation of an endangered capercaillie (*Tetrao urogallus*) population at the Southern edge of the species range. *Conservation Genetics,* 8, 659-670.

Rutledge, L.Y., Holloway, J.J., Patterson, B.R. & White, B.N. 2009. An improved field method to obtain DNA for individual identification from wolf scat. *The Journal of Wildlife Management,* 73, 1430-1435.

Segelbacher, G. 2002. Non-invasive genetic analysis in birds: testing reliability of feather samples. *Molecular Ecology Notes*, 2, 367-369.

Segelbacher, G. & Piertney, S. 2007. Phylogeography of the European capercaillie (*Tetrao urogallus*) and its implications for conservation. *Journal of Ornithology,* 148, S269-S274.

Segelbacher, G., Paxton, R.J., Steinbrück, G., Trontelj, P. & Storch, I. 2000. Characterisation of microsatellites in capercaillie *Tetrao urogallus* (AVES). *Molecular Ecology*, 9, 1919-1952.

Segelbacher, G., Höglund, J. & Storch, I. 2003. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. *Molecular Ecology*, 12, 1773-1780.

SNH, 2013. Capercaillie survey methods. https://www.nature.scot/sites/default/files/2018-01/Guidance-Licensing-Capercaillie-survey-methods.pdf

Solari, A.J. 1994. *Sex chromosomes and sex determination in vertebrates*. London CRC Press, pp 43-73.

Solberg, K.H., Bellemain, E., Drageset, O.-M., Taberlet, P. & Swenson, J.E. 2006. An evaluation of field and non-invasive genetic methods to estimate brown bear (*Ursus arctos*) population size. *Biological Conservation,* 128, 158–168.

Taberlet, P., Griffin, S., Goosens, B., Quesriau, S., Manceau, V., Escaravage, N., Waits, L.P. & Bouvet, J. 1996. Reliable geotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, 24, 3189-3194.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution,* 24,1596-1599.

Thompson, S.K. 1991. Adaptive cluster sampling: designs with primary and secondary units. *Biometrics* 47, 1103-1115.

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

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. 2004 Microchecker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4, 535-538.

Watson, A. & Moss, R. 2008. *Grouse.* London: Harper Collins.

Wilkinson, N.I., Langston, R.H.W., Gregory, R.D., Gibbons, D.W. & Marquiss, M. 2002. Capercaillie *Tetrao urogallus* abundance and habitat use in Scotland, in winter 1998-99. *Bird Study*, 49, 177-185.

Zhan, X., Li, M., Zhang, Z., Goossens, B., Chen, Y., Wang, H., Bruford, M.W. & Wei, F. 2006. Molecular censusing doubles giant panda population estimate in a key nature reserve. *Current Biology,* 16, R451–R452.

ANNEX 1 – GLOSSARY OF GENETIC ANALYSIS TERMS

Allele
The different, alternative forms of a gene that can exist at a single locus

Allelic Dropout
When an allele is not amplified in one individual and thus creates an apparent mismatch that is actually an artefact and the individual is more similar at that locus than appears from the genotype data.

DNA
A molecule that carries most of the genetic instructions used in the development, functioning and reproduction of all known living organisms and many viruses.

Gene
A locus (or region) of DNA that encodes a functional RNA or protein product, and is the molecular unit of heredity. The transmission of genes to an organism's offspring is the basis of the inheritance of phenotypic traits.

Genetic marker
A gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed in genetic data.

Genome
A genome is an organism's complete set of DNA, including all of its genes. Each genome contains all of the information needed to build and maintain that organism. In humans, a copy of the entire genome—more than 3 billion DNA base pairs—is contained in all cells that have a nucleus.

Genotype
An individual's collection of genes. The term also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the genes' DNA is used to make protein and RNA molecules.

Heterozygosity
A diploid organism is heterozygous at a gene locus when its cells contain two different alleles of a gene. The cell or organism is called a heterozygote specifically for the allele in question.

Locus (*pl.* loci)
The specific location or position of a gene, DNA sequence, on a chromosome

Microsatellite
A microsatellite is a tract of repetitive DNA in which certain DNA motifs (ranging in length from 2–5 base pairs) are repeated, typically 5-50 times. Microsatellites occur at thousands of locations in the human genome and they are notable for their high mutation rate and high diversity in the population.

Mitochondria
An organelle found in large numbers in most cells, in which the biochemical processes of respiration and energy production occur. DNA found within the mitochondrial (mtDNA) is usually inherited from the mother.

Nuclear
Relating to the nucleus of a cell.

Phylogeography
The study of the historical processes that may be responsible for the contemporary geographic distributions of individuals. This is accomplished by considering the geographic distribution of individuals in light of genetics, particularly population genetics.

Polymorphism
Natural variations in a gene, DNA sequence, or chromosome that have no adverse effects on the individual and occur with fairly high frequency in the general population

Polymerase Chain Reaction (PCR)
A technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Single Nucleotide Polymorphism (SNP, pronounced “snip”)
The most common type of genetic variation among people and many other organisms. Each SNP represents a difference in a single DNA building block, called a nucleotide.

ANNEX 2 – DETAILS OF FEATHER SAMPLES USED IN GENETIC ANALYSIS

WildGenes ID	Sample ID	Forest	Sample collection	DNA Extracted	PCR Amplifed	Genotyped >6 loci	Individual DNA Analysis ID	Field Sex	Molecular Sex	Notes (reason for exclusion)
CAP123	KF63	Kinveachy	brood count	y	y	y	KINV03	F	M	
CAP124	KF19	Kinveachy	brood count	y	y	y	KINV04	F	M	
CAP144	KF35	Kinveachy	brood count	y	y	y	KINV05		M	
CAP145	KF21	Kinveachy	brood count	y	y	y	KINV06	F	F	
CAP146	KF31	Kinveachy	brood count	y	y	y	KINV07		M	
CAP148	KF43	Kinveachy	brood count	y	y	y	KINV08		Failed	
CAP149	KF66	Kinveachy	brood count	y	y	y	KINV09	M	Failed	
CAP150	KF65	Kinveachy	brood count	y	y	y	KINV10		Failed	
CAP181	KF71	Kinveachy	brood count	y	y	y	KINV16	M	M	
CAP182	KF73	Kinveachy	brood count	y	y	y	KINV17	F	Failed	
CAP183	KF70	Kinveachy	brood count	y	y	y	KINV18	M	M	
CAP093	KF28	Kinveachy	brood count	y	y	y	KINV20		F	
CAP125	KF38	Kinveachy	brood count	y	y	y	KINV21		M	
CAP143	KF64	Kinveachy	brood count	y	y	y	KINV22		M	
CAP082	KF20	Kinveachy	brood count	y	y	y	KINV24	F	F	
CAP083	KF53	Kinveachy	brood count	y	y	y	KINV25	F	F	
CAP085	KF60	Kinveachy	brood count	y	y	y	KINV26	M	M	
CAP090	KF47	Kinveachy	brood count	y	y	y	KINV27		M	
CAP091	KF40	Kinveachy	brood count	y	y	y	KINV28		M	
CAP092	KF25	Kinveachy	brood count	y	y	y	KINV29		M	
CAP098	KF23	Kinveachy	brood count	y	y	y	KINV30	M	M	
CAP099	KF16	Kinveachy	brood count	y	y	y	KINV31		F	
CAP100	KF30	Kinveachy	brood count	y	y	y	KINV32		M	
CAP101	KF17	Kinveachy	brood count	y	y	y	KINV33	M	M	
CAP102	KF57	Kinveachy	brood count	y	y	y	KINV34		F	

WildGenes ID	Sample ID	Forest	Sample collection	DNA Extracted	PCR Amplifed	Genotyped >6 loci	Individual DNA Analysis ID	Field Sex	Molecular Sex	Notes (reason for exclusion)
CAP103	KF68	Kinveachy	brood count	y	y	y	KINV35	F	F	
CAP104	KF58	Kinveachy	brood count	y	y	y	KINV36	M	M	
CAP105	KF32	Kinveachy	brood count	y	y	y	KINV37		M	
CAP109	KF44	Kinveachy	brood count	y	y	y	KINV38		M	
CAP110	KF45	Kinveachy	brood count	y	y	y	KINV39		M	
CAP111	KF39	Kinveachy	brood count	y	y	y	KINV40		F	
CAP112	KF61	Kinveachy	brood count	y	y	y	KINV41	M	Failed	
CAP114	KF36	Kinveachy	brood count	y	y	y	KINV42		M	
CAP115	KF46	Kinveachy	brood count	y	y	y	KINV43		M	
CAP116	KF33	Kinveachy	brood count	y	y	y	KINV44		M	
CAP117	KF29	Kinveachy	brood count	y	y	y	KINV45		M	
CAP119	KF51	Kinveachy	brood count	y	y	y	KINV46		M	
CAP120	KF62	Kinveachy	brood count	y	y	y	KINV47	M	Failed	
CAP121	KF52	Kinveachy	brood count	y	y	y	KINV48		M	
CAP126	KF22	Kinveachy	brood count	y	y	y	KINV49	M	M	
CAP132	KF50	Kinveachy	brood count	y	y	y	KINV50		Failed	
CAP133	KF55	Kinveachy	brood count	y	y	y	KINV51		M	
CAP135	KF26	Kinveachy	brood count	y	y	y	KINV52		M	
CAP136	KF48	Kinveachy	brood count	y	y	y	KINV53		M	
CAP138	KF34	Kinveachy	brood count	y	y	y	KINV54		M	
CAP139	KF56	Kinveachy	brood count	y	y	y	KINV55		M	
CAP141	KF15	Kinveachy	brood count	y	y	y	KINV56	M	M	
CAP147	KF14	Kinveachy	brood count	y	y	y	KINV57	F	F	
CAP153	KF59	Kinveachy	brood count	y	y	y	KINV58	M	M	
CAP154	KF18	Kinveachy	brood count	y	y	y	KINV59	M	M	
CAP189	KF54	Kinveachy	brood count	y	y	y	KINV60	M	M	
CAP151	KF42	Kinveachy	brood count	y	y				M	
CAP185	KF69	Kinveachy	brood count	y	y			M	Failed	

WildGenes ID	Sample ID	Forest	Sample collection	DNA Extracted	PCR Amplifed	Genotyped >6 loci	Individual DNA Analysis ID	Field Sex	Molecular Sex	Notes (reason for exclusion)
CAP184	KF72	Kinveachy	brood count	y	y			F	Failed	
CAP094	KF24	Kinveachy	brood count	y					Failed	
CAP134	KF27	Kinveachy	brood count	y					M	
CAP118	KF37	Kinveachy	brood count	y					Failed	
CAP113	KF41	Kinveachy	brood count	y					Failed	
CAP137	KF49	Kinveachy	brood count	y					F	
CAP152	KF67	Kinveachy	brood count	y					Failed	
CAP002	KF13	Kinveachy	fence strike	y						Used as positive control
CAP191	KM3	Kinveachy	lek survey	y	y	y	KINV23	M	M	
CAP170	KF07	Kinveachy	spring transect	y	y	y	KINV01		F	
CAP171	KF08	Kinveachy	spring transect	y	y	y	KINV02		Failed	
CAP164	KF01	Kinveachy	spring transect	y	y	y	KINV11	F	F	
CAP165	KF02	Kinveachy	spring transect	y	y	y	KINV12		Failed	
CAP168	KF05	Kinveachy	spring transect	y	y	y	KINV13		M	
CAP173	KF10	Kinveachy	spring transect	y	y	y	KINV14		Failed	
CAP175	KF12	Kinveachy	spring transect	y	y	y	KINV15		Failed	
CAP172	KF09	Kinveachy	spring transect	y	y	y	KINV19		Failed	
CAP166	KF03	Kinveachy	spring transect	y	y				Failed	
CAP167	KF04	Kinveachy	spring transect	y	y				Failed	
CAP174	KF11	Kinveachy	spring transect	y	y				Failed	
CAP169	KF06	Kinveachy	spring transect							too small
CAP084	RF21	Rothiemurchus	brood count	y	y	y	ROTH03	F	F	
CAP086	RF20	Rothiemurchus	brood count	y	y	y	ROTH04		Failed	
CAP087	RF18	Rothiemurchus	brood count	y	y	y	ROTH05	M	M	
CAP088	RF15	Rothiemurchus	brood count	y	y	y	ROTH06	M	M	
CAP107	RF19	Rothiemurchus	brood count	y	y	y	ROTH07	M	M	
CAP108	RF16	Rothiemurchus	brood count	y	y	y	ROTH08	F	M	
CAP122	RF14	Rothiemurchus	brood count	y	y	y	ROTH09	M	M	

WildGenes ID	Sample ID	Forest	Sample collection	DNA Extracted	PCR Amplifed	Genotyped >6 loci	Individual DNA Analysis ID	Field Sex	Molecular Sex	Notes (reason for exclusion)
CAP129	RF12	Rothiemurchus	brood count	y	y	y	ROTH10	F	F	
CAP131	RF17	Rothiemurchus	brood count	y	y	y	ROTH11	F	M	
CAP095	RF24	Rothiemurchus	brood count	y	y	y	ROTH21	F	F	
CAP096	RF31	Rothiemurchus	brood count	y	y	y	ROTH22	F	F	
CAP097	RF32	Rothiemurchus	brood count	y	y	y	ROTH23	F	F	
CAP142	RF25	Rothiemurchus	brood count	y	y	y	ROTH25	M	F	
CAP186	RF28	Rothiemurchus	brood count	y	y	y	ROTH26	F	F	
CAP187	RF27	Rothiemurchus	brood count	y	y	y	ROTH27	M	M	
CAP140	RF22	Rothiemurchus	brood count	y	y	y	ROTH24		M	
CAP089	RF10	Rothiemurchus	brood count	y	y			F	Failed	
CAP128	RF11	Rothiemurchus	brood count	y				M		No genotyping, low DNA concentration
CAP130	RF13	Rothiemurchus	brood count	y				F		No genotyping, low DNA concentration
CAP106	RF23	Rothiemurchus	brood count	y				M	Failed	
CAP127	RF30	Rothiemurchus	brood count	y				M	F	
CAP180	RF26	Rothiemurchus	brood count					M		
CAP179	RF29	Rothiemurchus	brood count					M		
CAP177	RC8	Rothiemurchus	lek survey	y	y	y	ROTH01	M	M	
CAP178	RS6	Rothiemurchus	lek survey	y	y	y	ROTH02		Failed	
CAP192	RM14	Rothiemurchus	lek survey	y	y	y	ROTH18	M	M	
CAP193	RM22	Rothiemurchus	lek survey	y	y	y	ROTH19	M	M	
CAP194	RM23	Rothiemurchus	lek survey	y	y	y	ROTH20	M	M	
CAP188	RC7	Rothiemurchus	lek survey	y	y			F	Failed	
CAP081	RC4	Rothiemurchus	lek survey					M		
CAP155	RF03	Rothiemurchus	spring transect	y	y	y	ROTH12	F	F	
CAP156	RF08	Rothiemurchus	spring transect	y	y	y	ROTH13		M	
CAP158	RF01	Rothiemurchus	spring transect	y	y	y	ROTH14		M	
CAP162	RF06	Rothiemurchus	spring transect	y	y	y	ROTH15		M	

WildGenes ID	Sample ID	Forest	Sample collection	DNA Extracted	PCR Amplifed	Genotyped >6 loci	Individual DNA Analysis ID	Field Sex	Molecular Sex	Notes (reason for exclusion)
CAP163	RF09	Rothiemurchus	spring transect	y	y	y	ROTH16		M	
CAP161	RF07	Rothiemurchus	spring transect	y	y	y	ROTH17		Failed	
CAP159	RF02	Rothiemurchus	spring transect	y	y				Failed	
CAP160	RF04	Rothiemurchus	spring transect	y	y				F	
CAP157	RF05	Rothiemurchus	spring transect							No calamus

www.nature.scot

ISBN: 978-1-78391-383-1

Policy and Advice Directorate, Great Glen House,
Leachkin Road, Inverness IV3 8NW
T: 01463 725000

You can download a copy of this publication from the SNH website.

recycle

Scottish Natural Heritage
Dualchas Nàdair na h-Alba
All of nature for all of Scotland
Nàdar air fad airson Alba air fad