

Sample diversity adds value to non-invasive genetic assessment of a pine marten (*Martes martes*) population in Galloway Forest, southwest Scotland

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Abstract The European pine marten (*Martes martes*) is a species of considerable conservation interest in Britain due to its rarity and status as a recovering native carnivore. In recent years, there has been increased application of non-invasive genetic sampling methods in population studies of *Martes* species. We investigated the effect of sample source (hair and faeces) in the non-invasive assessment of the distribution, population size and density of pine martens in the Fleet Basin in Galloway Forest, southwest Scotland. Fifty-two hair samples and 114 scats were collected during September and October 2014. Genetic analysis was used to identify the species, gender and individual genotype of samples. There was a significant difference in the genotyping success rate for hair samples (43 %) and scat samples (24 %). In total, 15

individual pine martens were identified; 7 males and 8 females. Capture-recapture programme Capwire produced a population size estimate of 18 individuals (95 % CI 15 to 25). Of the 15 individuals sampled, 14 (93 %) were detected from scat sampling and 5 (33 %) were detected from hair sampling. The population density estimate for a post-breeding population was 0.13 to 0.15 pine martens per square kilometre which is towards the lower limit of densities reported for pine martens elsewhere in Scotland. Data from the study highlight that future non-invasive studies aiming to determine pine marten population size and density should incorporate the collection of both hair and scat samples in order to detect as many individuals within the population as possible.

Keywords Pine marten · *Martes martes* · Non-invasive genetic sampling · Population size · Population density

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Introduction

The European pine marten (*Martes martes*) is a species of considerable conservation interest in Britain due to its rarity and status as a recovering native carnivore. The pine marten was historically widespread in Britain (Langley and Yalden 1977) and was the second commonest carnivore during the Mesolithic (Maroo and Yalden 2000). Woodland clearance and persecution associated with predator control for gamekeeping and trapping for fur caused a severe population decline during the 18th and 19th centuries, and the population became confined to a stronghold in the northwest of Scotland and restricted areas of northern England and Wales by 1900 (Langley and Yalden 1977). Today, the population is recovering and expanding its range in Scotland (Croose et al. 2013) but has not recovered from its decline in England and Wales and only occupies a restricted part of its historical range (Birks

and Messenger 2010). As the population expands its range and re-colonises areas from which it has been absent for significant periods of time, it is essential to have reliable and cost-effective methods that can be implemented to monitor the population.

Monitoring species distribution and abundance is a fundamental issue in the conservation and management of wildlife populations (Riddle et al. 2003; Wilson and Delahay 2001). Data on species are required to fulfil reporting obligations to The Convention on the Conservation of European Wildlife and Natural Habitats (the Bern Convention) (JNCC 2013; Council of Europe 2014) and to inform the assessment of species status for conservation programmes such as the International Union for Conservation of Nature (IUCN) Red List (Couturier et al. 2013). Monitoring programmes are typically required when enhancing populations of endangered species; assessing the progress of a reintroduction or recovery programme; reducing the population size of a pest species; defining the biological diversity or ecological health of an area and understanding the effects of land use and management practices on a particular species or community (Wilson and Delahay 2001; Witmer 2005).

Gathering accurate data for many species, particularly carnivores, can be problematic as they are often nocturnal, elusive and secretive, occur at low densities and may occupy large home ranges (Wilson and Delahay 2001; Mills et al. 2000; Riddle et al. 2003). In recent years, there has been increased application of non-invasive genetic sampling to gather data on a range of population parameters, including distribution, abundance and population density that can inform wildlife conservation management strategies (Waits and Paetkau 2005). Non-invasive sampling and genetic tagging is advantageous because it does not necessitate capturing animals (thus avoiding the stress and additional mortality associated with trapping); reduces bias resulting from trap response; can increase the number of observations and thus improve population estimates (Miller et al. 2005) and is less resource (time and cost) consumptive than live trapping or radio telemetry. Non-invasive methods have been applied in several population studies of *Martes* species, including American martens (*M. americana*) (Mowat and Paetkau 2002), fishers (*M. pennanti*) (Zielinski et al. 2006; Williams et al. 2009), stone martens (*M. foina*) (Pilot et al. 2007; Ruiz-González et al. 2008; Ruiz-González et al. 2013) and pine martens (Mullins et al. 2010; Sheehy et al. 2013; O'Mahony et al. 2014; Kubasiewicz 2014). Research has also investigated the effects of sample source (hair and faecal samples) and diet on amplification success of DNA and genotyping success rates (Broquet et al. 2007).

In the early 1980s, pine martens were reintroduced to Galloway Forest in southwest Scotland to re-establish a population in southern Scotland where martens had been extirpated (Shaw and Livingstone 1994). Twelve pine martens were

released at two separate sites 10 km apart, comprising six animals at each release site (Shaw and Livingstone 1994). Although the released animals were not monitored, a dearth of subsequent sightings suggested that one of the releases was unsuccessful (Shaw and Livingstone 1994). Therefore, it is possible that the pine marten population in Galloway Forest was founded on just six animals.

In this study, we investigated the effect of sample source (hair and faeces) in the non-invasive assessment of the distribution, population size and density of pine martens within part of Galloway Forest.

Materials and methods

Study area

The study area was located in the Fleet Basin, at the south-eastern edge of Galloway Forest in southwest Scotland, United Kingdom (55° 02' N, 4° 16' W) (see Fig. 1). The region has a mild climate with average temperatures ranging from 1.3 °C in the winter to 19.5 °C in the summer (Met Office 2013) and average annual rainfall of around 1600 mm (Forest Enterprise 2012). The Fleet Basin is a commercial conifer plantation covering approximately 100 km² and is dominated by Sitka spruce (*Picea sitchensis*) and Lodgepole Pine (*Pinus contorta*), with minor coverage of larch (*Larix spp.*), Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and Douglas fir (*Pseudotsuga menziesii*), and very little broadleaf (Forest Enterprise 2012). The dominant tree age structure is pole stage and mature and old forest stage crops with a smaller proportion at establishment or thicket stage (Forest Enterprise 2012). Evidence of disease caused by *Phytophthora ramorum* was confirmed on larch in the forest in 2011 (Forestry Commission Scotland 2013) and consequently, sanitation felling of larch was carried out in the Fleet Basin during the study period, in order to contain outbreaks and spread of the pathogen. The Fleet Basin is designated as a Red Squirrel Stronghold, whereby management is driven by the requirements of red squirrel (*Sciurus vulgaris*) conservation.

Sample collection

Sample collection was undertaken during September and October 2014. At this time of year, the pine marten population was at a post-breeding annual peak as juveniles born during the spring would still have been present within their mother's territory prior to dispersal during the winter (Larroque et al. 2014). Hair tubes, comprising lightweight polyvinyl chloride tubes measuring 250 mm in length and 118 mm in width, were used to gather hair samples (for full details, see Mullins et al.



Fig. 1 Location of the study area (black square) and pine marten distribution (grey shaded) in Scotland (inset). Location of hair tubes (black circles) and scat transects (grey lines) within the study area

2010). A total of 100 hair tubes were placed at a density of one tube per 1 km² Ordnance Survey grid square throughout the study area, with approximately 1 km spacing between each tube where practicable (see Fig. 1). Grid squares that had less than 50 % forest cover and those without vehicle tracks for easy access were excluded. Hair tubes were installed within 10 m of the forest track and were fixed vertically to the trunk of a tree using wire, approximately 1 m off the ground. Two patches of ‘mouse glue’ (Pest Control Supermarket, <http://www.pestcontrolsupermarket.com>) cut to 1 cm × 1.4 cm rectangles and stuck with double-sided sticky tape to blocks of 4-mm thick Correx Board measuring 2 cm × 2.5 cm were fitted to the base of the tube to catch hair when an animal entered. Tubes were baited with chicken meat attached to the inside of the tube lid with wire or string. Four sampling sessions were conducted, with tubes sampled between 5 and 9 days apart over a total of 32 days. During each sampling session, any glue patches that had collected a hair sample were removed and placed in a plastic sample pot, labelled with a unique sample number and subsequently frozen at -4 °C within 12 h of collection. Fresh glue patches were fitted and fresh bait was installed in the tube on each occasion when patches were removed.

Pine marten scats (faeces) were collected from 32 transects on forest tracks, measuring between 1 and 4.45 km, and totalling 43.5 km, distributed throughout the study area (see Fig. 1). Scats were also collected on an ad hoc basis whilst installing and sampling hair tubes. On collection, each individual scat was placed into a zip-locked plastic bag with a unique sample number and subsequently frozen at -4 °C within 12 h of collection.

DNA analysis

Genomic DNA was isolated from the hair samples using the ZR Genomic DNA™-Tissue MicroPrep (D3041) kit (ZYMO Research, CA, USA) using the protocol for hair DNA extraction (D3040). For DNA isolation from scats, approximately 0.2 g of scat material was transferred to 1 ml Stool Transport and Recovery buffer (S.T.A.R.®, Roche) in a 2-ml microfuge tube. The tube was vortexed for 15 s and then left to stand for at least 60 min. Supernatant (200 µl) was transferred to a microcentrifuge tube for DNA isolation. DNA was isolated using the ZR Genomic DNA™-Tissue MicroPrep (D3041) kit (ZYMO Research, CA, USA). Real-time quantitative PCR (qPCR) assays for species and sex identification were carried out as described in Mullins et al. (2010). For species identification, an 85-bp fragment of the mitochondrial DNA control region was amplified using the primers PM3F (5'-CTTGCCCCATGCATATAAGCA-3') and PM-REV2 (5'-GCCTGGTGATTAAGCTCGTGAT-3'), and pine marten samples were identified with the hybridization of a species-specific TaqMan™ MGB® probe (PM3, 5'-6-FAM-GTGACCTCACTTAG-MGB-3'). For sex determination multiplex, PCR targeting the ZFX and ZFY genes was carried out with TaqMan™ MGB® labelled probes MMX, (5'-6-FAM-TGTGTCTCTCTGTCAAMGB-3') and MMY, (5'-VIC-CCTGGTCTGAAAACCT-MGB-3') and primers MMXF, (5'-GGCAGAGCAACCCTGTCATAA-3'); MMXR, (5'-GGGCCTGAGGTTGGTACCACCA-3'); MMYF, (5'-GCATTGGGCTCCCTGCT-3') and MMYR, (5'-AGATATCCAAATACATGTGGCTTTAAATG-3'). Two PCR replicates were carried out for molecular sexing (Lynch et al. 2006). Females were identified through the amplification of ZFX only, while a signal from both ZFX and ZFY probes indicated male DNA was amplified. The ZFX allele therefore acted as an internal amplification control for the assay. Samples were selected for genotyping based on the results of the qPCR sex-typing assay with samples with a ZFX C_T value <34 selected for genotyping.

Microsatellite analysis to identify individual pine marten was carried out using eight microsatellite markers. These were as follows: Gg7; Ma2; Mel105; Mvi1341; Mvis075 (see Mullins et al. 2010) and Mar21; Mar53 and Mar08 (Natali et al. 2010). Each sample was analysed in duplicate and only samples giving identical results in the replicates were scored. PCR reactions were carried out in three multiplex reactions. Multiplex 1 contained Gg7 and Mvi1341; Multiplex 2 contained Mar21, Mar53 and Ma2 and Multiplex 3 contained Mel105, Mar08 and Mvis075. Each PCR reaction contained 5-µl GoTaq Hotstart master mix (Promega Inc.), 1-µl multiplex primer mix (200 nM each primer) and 4-µl DNA extract. The PCR protocol was 95 °C initial denaturation for 5 min, followed by 40 cycles of 94 °C for 30 s, 58 °C for 1 min and 72 °C for 30 s, with a final extension time of 30 min at 72 °C.

Fragment analysis was carried out on an ABI PRISM 310 genetic analyser under standard run conditions with 4 % polyacrylamide. Alleles were scored against a GS500 LIZ™ size standard using GeneMapper software version 3.7 (Applied Biosystems). Genotype data were analysed for probability of identity (PI and PIsibs), observed (H_o) and expected (H_e) heterozygosity and allele frequencies using GENALEX version 6 (Peakall and Smouse 2006).

Population size and density estimates and statistical analysis

A population size estimate was derived using the capture-recapture programme Capwire (Miller et al. 2005). Capwire is appropriate for estimating population size in non-invasive genetic studies as it allows for sampling with replacement and takes account of multiple observations of an individual within a sampling session. Capwire also takes account of the capture heterogeneity within the sampled population by applying the two-innate rates model to assign a ‘capture type’ to individuals (a high or low probability of being captured) (Miller et al. 2005). Capwire has been shown to provide accurate population estimates when analysing small populations with substantial capture heterogeneity (Miller et al. 2005). All captures of individual pine martens, identified through genotyping, were grouped into a single sampling session scheme for analysis. The maximum population size was set as 100, and 95 % confidence intervals were used to calculate a population estimate with 1000 parametric bootstrap replicates. The likelihood ratio test (LRT) was implemented to choose the most suitable model for analysis; the even capture model (ECM), which assumes that there is no capture heterogeneity and that each individual has an equal chance of being captured, or the two-innate rates model (TIRM), which assigns a high or low capture probability to individuals.

To estimate the population density, an effective trapping area was estimated, with the inclusion of a buffer strip, to account for ‘edge effects’ caused by movement of animals in and out of the area (Otis et al. 1978; Royle et al. 2013). For the study area, a convex hull was delineated by the locations of the outermost hair tubes and scat transects using MapInfo Professional (v12.0). The maximum distance moved was calculated for individual pine martens that were detected at a

minimum of two locations ($n=9$), and a straight line was drawn between the two detection locations that were the furthest apart using MapInfo. The mean maximum distance moved for all of these individuals was calculated to provide an estimator of home range diameter (mean = 2.42 km), and the width of half mean maximum distance moved (1,021 m) was applied to the study area convex hull to create an effective trapping area (Royle et al. 2013). Lochs and significant areas of open ground, which mostly comprised moorland, were excluded from the effective trapping area as pine martens strongly avoid this type of habitat (Balharry 1993; Caryl et al. 2012). The minimum area of open ground that was excluded was 1.37 km². Population density was calculated by dividing the population size by the effective trapping area (Otis et al. 1978). Two post-breeding population density estimates were produced: one based on the minimum population size as derived from individual genotypes and one based on the population size estimate determined by Capwire.

Fisher’s exact test was used to test for statistical significance in the genotyping success rate for hair samples and scat samples.

Results

Sample collection and DNA analysis

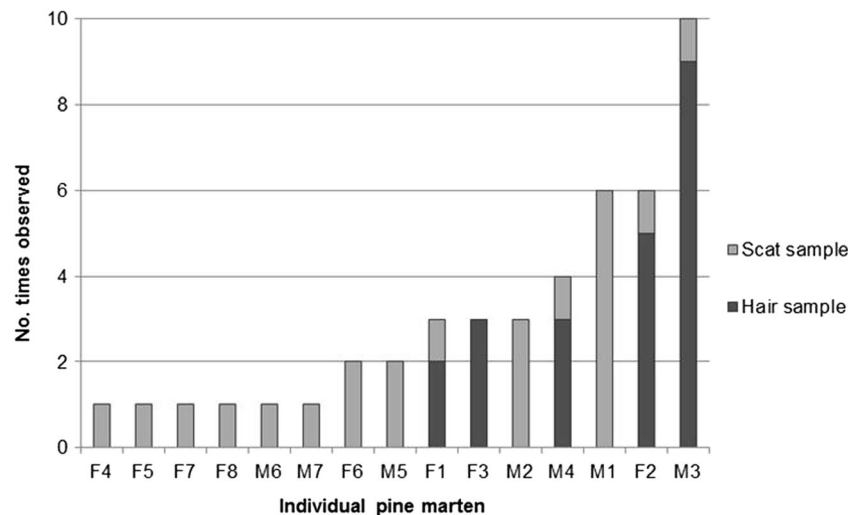
In total, 52 hair samples were collected from the hair tubes. Hair tube visitation rate increased across the sampling sessions from 4 % in the first sampling session, 10 % in the second sampling session, 13 % in the third sampling session to 25 % in the fourth sampling session; thus 6.3 times as many samples were collected in the fourth sampling session compared with the first. Of the 52 hair samples, 98 % ($n=51$) were identified as pine marten by DNA analysis, of which 88 % ($n=46$) were gender-typed (30 male samples and 16 female samples) (see Table 1). There were almost twice as many male detections as female detections in the hair tubes; however, genotyping revealed that this was biased by one individual male that was detected in hair tubes nine times, and the number of individual females detected ($n=3$, 60 %) in hair tubes was actually higher than individual males ($n=2$, 40 %). Of the

Table 1 The results of the DNA analysis of hair and scat samples

| | Samples collected | Samples identified as pine marten | Samples determined to gender ^a | No. male/female detections | Samples determined to genotype ^a | No. individual genotypes (males/females) |
|-------|-------------------|-----------------------------------|---|----------------------------|---|--|
| Hair | 52 | 51 (98 %) | 46 (88 %) | 30:16 | 22 (43 %) | 5 (2:3) |
| Scat | 114 | 96 (84 %) | 83 (86 %) | 39:44 | 23 (24 %) | 14 (7:7) |
| Total | 166 | 147 (89 %) | 129 (88 %) | 69:60 | 45 (31 %) | 15 (7:8) |

^a The percentage is of the samples confirmed as pine marten

Fig. 2 Detections of individual pine martens in scat and hair samples, in ascending order. Males are identified as M# and females as F#



hair samples, 43 % ($n=22$) were genotyped, identifying five individual pine martens: two males and three females. The number of times an individual pine marten was detected in a hair tube varied from two to nine (average=4.4) (see Fig. 2).

A total of 114 scats was collected, of which 84 % ($n=96$) were identified as pine marten by DNA analysis. Of these scats, 86 % ($n=83$) were gender-typed (39 male samples and 44 female samples) and 24 % ($n=23$) were genotyped, identifying a total of 14 individual pine martens: seven males and seven females (see Table 1). The number of times an individual pine marten was detected in scats varied from one to six (average=1.6), although the majority of individuals (71 %, $n=10$) were only detected once (see Fig. 2).

In total, individual genotypes were identified for 31 % ($n=45$) of hair and scat samples collectively. There was a significant difference ($p=0.02$, Fisher's exact test) in the genotyping success rate for hair samples (43 %) and scat samples (24 %) (see Table 1). All individuals except one (93 %, $n=14$) were detected in scats, whereas only 33 % ($n=5$) of individuals were detected in hair samples (see Fig. 2). Twenty-seven percent ($n=4$) of individuals were identified in both scat and hair samples. The number of times an individual was detected ranged from one to ten (average=three), with the majority of individuals (73 %, $n=11$) detected less than four times (see Fig. 2). There was no difference in the gender of individuals identified in either hair or scat samples (2 males: 3 females for hairs; 7 males: 7 females for scats), but the small sample size prevents further analysis on this.

All eight microsatellite loci were polymorphic but all had only two alleles except for Gg7 which had three alleles. The probability of identity (PI) using all eight loci was $PI=0.00069$ and $PI_{sibs}=0.024$. The mean expected heterozygosity (H_E) was 0.44, and the mean observed heterozygosity (H_O) was 0.48. Only one loci, Gg7, showed a significant deviation from Hardy-Weinberg expectations ($p=0.0339$) although this may not be significant due to the small sample size.

Pine marten population size, density and composition

A total of 15 individual pine martens were identified through genotyping hair and scat samples, comprising seven males and eight females; a sex ratio of 88:100. This represents a post-breeding population at an annual peak, because the kits born in the spring prior to this study would still have been within their mother's territory prior to dispersing during the winter (Larroque et al. 2014), thus temporarily inflating the population size. The LRT was implemented in Capwire and selected the TIRM to analyse the data, based on individual

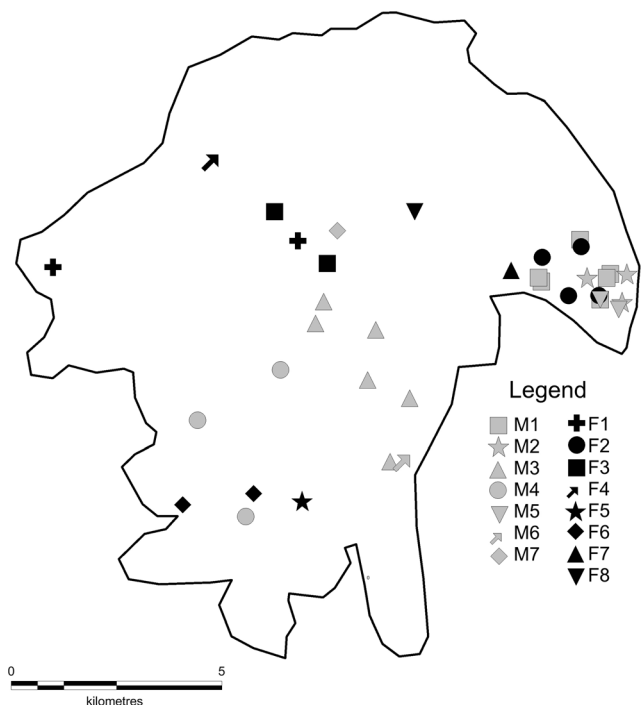


Fig. 3 The distribution and composition of individual genotyped pine martens in the Fleet Basin. Males are identified as M# and females as F#

heterogeneity in the capture data ($p=0.002$). Capwire generated a population size estimate of 18 (95 % CI 15 to 25).

The population density estimates were determined as 0.13 martens per km² for the minimum population size (based on 15 individuals) and 0.15 martens per km² for the Capwire population estimate (based on 18 individuals). As these estimates represent a post-breeding population including pre-dispersal juveniles, the density for adults occupying stable home ranges throughout the year will be lower than the densities derived.

The maximum distance moved per individual pine marten detected at a minimum of two locations ($n=9$) ranged from 0.49 to 5.84 km. A particularly long distance moved by a female was 5.84 km that may have been a juvenile making pre-dispersal or dispersal movements. The average distance moved for all individuals excluding this female was 1.99 km; the average distance moved for a male ($n=5$) was 2.18 km and for a female, excluding the individual that moved the longer distance ($n=3$), was 1.69 km.

The distribution and composition of genotyped individuals is shown in Fig. 3.

Discussion

Efficacy of non-invasive sampling methods

This study is the first in Britain to use genotyping of both hair and scat samples to determine the size, density and composition of a pine marten population. Only 33 % of individuals were detected via hair samples, yet all individuals except one (93 %) were detected via scats. Despite hair sampling being used as an established method for studying pine martens, it has been demonstrated during other studies that some pine martens will not use hair tubes (PT, unpublished observation; Kubasiewicz 2014; J. Power, *pers. comm.*). Because only one third of the individuals were detected in hair tubes in this study, if hair tubes alone had been used for sampling without incorporating data from scats, two thirds of the individuals would not have been detected, thus omitting crucial information on the population. The majority of recent non-invasive studies of pine marten population size and density have used sampling and genotyping of hairs only and have not included scats (Lynch et al. 2006; Sheehy et al. 2013; O'Mahony et al. 2014; Kubasiewicz 2014); these could result in underestimating population size and density by not detecting pine martens that do not use hair tubes. Scat sampling is a relatively simple method that requires few resources because only a single sampling session is needed, and it can easily be carried out concurrently whilst sampling hair tubes. Nevertheless, scats have a significantly lower genotyping success rate than hairs do (24 % compared with 43 % in this study). Data from this study highlight the importance of sampling and genotyping both hair and scat samples for a

comprehensive survey to detect as many individual animals as possible.

The proportion of hair tubes visited by pine martens increased during the study, from 4 % in the first sampling session to 25 % in the final sampling session, which is consistent with previous hair tube studies (O'Mahony et al. 2014; Kubasiewicz 2014). This increase is likely due to the martens having more time to locate the hair tubes, particularly if they have large home ranges which may encompass several tubes, or because tubes were located in an infrequently visited part of a home range. Of the individual pine martens detected via hair samples, 80 % visited at least one hair tube twice, and eight different hair tubes were visited twice by the same individual. This suggests that once an individual has located and visited a hair tube, it may continue to visit it multiple times. It is possible that if the hair tubes were left in situ for longer, the proportion of tubes visited by pine martens would have increased; however, no individuals were detected for the first time in the final sampling session, which justifies not extending the survey period and the number of sampling sessions.

There were almost double the number of male detections ($n=30$) when based on gender alone in hair samples; however, genotyping showed that this was biased by one individual male that was detected in hair tubes nine times and the number of individual females detected ($n=3$, 60 %) in hair tubes was actually higher than individual males ($n=2$, 40 %). Overall, there was little difference in the sex ratio of scats collected; 47 % of scats that were gender-typed were male and 53 % were female. The samples that were successfully genotyped showed a sex ratio of 1:1, suggesting that the scat collection method does not involve a sampling bias.

DNA analysis

The proportion of hairs and scats confirmed as pine marten by DNA analysis (98 and 84 %, respectively) was relatively high, as was the proportion of pine marten samples subsequently determined to gender (88 % for hairs, 86 % for scats). The genotyping success rate for hair samples in this study (43 %) was considerably lower than that achieved in other non-invasive studies of pine marten populations where the same molecular methods were used; 54 % in O'Mahony et al. (2014), 66 % in Sheehy et al. (2013), 72 % in Kubasiewicz (2014) and 92 % in Mullins et al. (2010). This could account for the proportionately low number of individuals ($n=5$) detected via the hair tubes compared with via scats; it is probable that additional individuals used the hair tubes but the hair samples were of insufficient quality to yield a genotype. During hair sampling, many of the glue patches became saturated with flies attracted to the meat bait, which likely prevented collection of good quality hair samples. Genotyping success rates are known to increase with the number of hairs and hair follicles in a sample (Mowat and Paetkau 2002; O'Mahony et

al. 2014; Kubasiewicz 2014) and are significantly greater with samples comprising more than ten hairs (O'Mahony et al. 2014). The genotyping success rate for scats in this study (24 %) was favourable. This may be due to the long period of dry weather experienced during the study, because DNA amplification success rates from faeces are highest when climatic conditions are dry (Murphy et al. 2007), and PCR amplification rates of carnivore scats decline significantly when scats are exposed to wet weather (Farrell et al. 2000).

Survey limitations

Establishing accurate population size and density estimates is problematic due to incomplete detection and the variable detection probabilities of different individuals (Efford et al. 2004, 2009). In this study, there was considerable capture heterogeneity within the population, as indicated by the Capwire analysis, with the number of detections of an individual marten ranging from one to ten. Variations in capture rate of different individuals may be influenced by gender, behaviour, age (adult or juvenile), status (whether individuals are territorial or non-territorial, or resident animals or dispersing) and the position of the detection device within the individual's home range (Belbachir et al. 2015). Some of the individuals identified, particularly those detected towards the periphery of the study area and those which were detected only once, may not have occupied fixed home ranges within the Fleet Basin and may have had home ranges which lie partly or mostly outside the study area. In this study, the average observation per individual pine marten was three. Miller et al. (2005) recommend that an average of three observations per individual is necessary for Capwire to achieve population size estimates that are within 10 % of the true population size, thus suggesting that the Capwire population estimate of 18 individuals is likely to be close to (within 10 % of) the true population size.

In non-invasive genetic studies, the samples not determined to genotype (57 % of hairs and 76 % of scats in this study) present particular difficulties. Of the non-genotyped samples, it is likely that the majority represent individuals genotyped via other samples, particularly when found in close proximity to genotyped samples; but when non-genotyped samples were found at greater distances from genotyped samples, it is more likely that they represent an unidentified individual. In this study, the presence of several non-genotyped but gender-typed samples located some distance from genotyped individuals suggests that there may have been an additional three unidentified individuals in the study area; one female and two males, located 3.6, 4.1 and 4.9 km from the nearest genotyped samples of the same gender, respectively. Interestingly, if there were three additional individuals in the study area that had not been identified through genotyping, this would bring the total population to 18 individuals, which

matches the population estimate derived from Capwire. Nevertheless, it is possible that some of these non-genotyped samples could simply be from genotyped individuals that were travelling large distances, either because they occupied large home ranges or were juveniles making pre-dispersal forays.

This study was conducted during September and October, when juvenile pine martens born during the spring would have been present in their mother's territory prior to dispersal during the winter (Larroque et al. 2014); hence, the population is at a post-breeding annual peak. Caution must be taken when interpreting data collected at this time of year, as it may result in overestimating the true population size and density of the adult population occupying stable home ranges during the rest of the year. In order to provide a more realistic picture of pine marten population size and density and avoid sampling pre-dispersal juveniles, the preferred time window for sampling is February to June, after juveniles from the previous year have dispersed and before juveniles born in that year have emerged from natal dens and become mobile.

Pine marten population size and density

The pine marten population density estimate for the Fleet Basin of 0.13–0.15 pine martens per km² for a post-breeding population is within the range of estimates observed for pine martens in Scotland, but is towards the lower limit. Population densities reported elsewhere in Scotland have ranged from 0.030–0.094 martens per km² in Minnoch in Galloway Forest (Bright and Smithson 1997) to 0.58 martens per km² in Novar in Ross-shire (Halliwell 1997). It has been suggested that the lower population density of pine martens in Galloway Forest is likely due to poorer quality habitat and lower food availability which may adversely affect carrying capacity (Bright and Smithson 1997). The Fleet Basin plantation is less than 60 years old (Forest Enterprise 2012). Young plantations are unlikely to offer suitable tree cavities for martens to den in, although potential den sites could be located elsewhere; for example, snagged branches of wind-blown trees, bird nests, root plates or cavities in tree roots. In a review of pine marten dens in Galloway Forest, only one arboreal cavity den was reported out of 85 dens detected and 84.7 % of dens detected were found in relatively exposed positions, such as snagged branches or bird nests (Birks et al. 2005). Martens occupying dens in exposed positions face extra energetic costs compared with those denning in arboreal cavities and the lack of suitable dens, particularly for breeding females, may limit breeding success, especially in commercial woodlands (Birks et al. 2005). Furthermore, pine marten population density is limited by abundance and availability of rodents (Zalewski and Jędrzejewski 2006). Vole survey transects undertaken in the Fleet Basin in 2014 prior to the study indicate that vole numbers were high and probably at or near maximum (JB, unpublished data), thus providing abundant availability of one of the

pine marten's dominant prey and suggesting that rodent availability should not limit pine marten population density in this area.

Conservation implications and recommendations

Previous studies using non-invasive genetic sampling of pine martens have been on populations at relatively high population density (2 martens per km² in Mullins et al. 2010; 1.01 to 4.42 martens per km² in Sheehy et al. 2013). This study demonstrates that non-invasive sampling methods offer an effective means of obtaining a range of data on pine marten populations at a low density, including species distribution, population size, density and composition, which can inform a variety of conservation and management strategies. Therefore, these methods can be applied for populations at lower densities or those at the early stages of establishment (i.e. during initial re-colonisation of an area or following a translocation). Results from this study highlight the importance of using a combined sampling approach, comprising both hair and scat samples, to maximise the comprehensiveness and accuracy of the data. Therefore, future studies aiming to determine pine marten population size and density through non-invasive sampling should incorporate the collection of both hair and scat samples in order to detect as many individuals within the population as possible. Of particular conservation interest is the question of how sanitation felling of larch to contain outbreaks of *Phytophthora ramorum* may affect pine marten home ranges, population size and density. This issue could be investigated using non-invasive sampling.

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