Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 28 (2): 265–271, 2017
OPEN ACCESS
Available online at:
http://www.italian-journal-of-mammalogy.it
doi:10.4404/hystrix-00007-2017

Research Article

# Non-invasive genotyping and spatial mark-recapture methods to estimate European pine marten density in forested landscapes

Laura M. Kubasiewicz[1,*], Chris P. Quine[2], Ron W. Summers[3], Rob Coope[2], Joan E. Cottrell[2], Stuart W. A'Hara[2], Kirsty J. Park[1]

[1] *Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA*
[2] *Forest Research, Centre for Ecosystems, Society and Biosecurity, Northern Research Station, Scotland, UK*
[3] *Royal Society for the Protection of Birds , Etive House, Beechwood Park , Inverness, UK*

*Keywords:*
non-invasive genotyping
elusive species
SECR modelling
population density
forest fragmentation

*Article history:*
Received: 31 August 2016
Accepted: 12 June 2017

*Acknowledgements*
We would like to thank David Bavin, Lizzie Croose, Tara Curry, Melissa Simmons and Kayleigh McCrory for help in the field; Jeremy Roberts, Shaila Rao, Ben Clinch and David Anderson for access to field sites; Bridget Laue (Forest Research) and Catherine O'Reilly (Waterford Institute of Technology, Ireland) for help and advice on genetic analysis. Funding was provided by the University of Stirling, Forestry Commission, Forest Research, the Royal Society of the Protection of Birds and Scottish Natural Heritage.

**Abstract**

Accurate wildlife population density estimates are important for conservation purposes, but can be difficult to obtain where species are elusive or rare. We use individual genotypes derived from hair samples and Spatially Explicit Capture Recapture (SECR) models to estimate the population density of European pine marten (*Martes martes*) and examine the effects of forest fragmentation on population size. We take the first steps towards linking the number of scats in an area to population density, which may eventually negate the need for expensive genetic analyses in the future. Population density estimates ranged from 0.07 km$^{-2}$ (95% CI 0.03–0.16) to 0.38 km$^{-2}$ (95% CI 0.11–1.07), which were mid to low compared to other estimates from Scotland. We found support for the previous finding that pine marten density in Scotland increases with forest fragmentation up to a threshold level (20–35% forest cover), beyond which it decreases. We found a non-linear relationship between scat counts and population density, although this relationship may be biased by factors not included in the analysis and should be viewed with caution. Following the recent re-inforcement of pine martens to Wales, non-invasive genetic sampling for population estimation may provide an effective way of monitoring their progress.

## Introduction

Robust measures of population density are a key component in wildlife conservation, for example to assess the effects of human activities on vulnerable species (Remis and Kpanou, 2011) or set sustainable quotas for game species (Brøseth et al., 2012). Non-invasive sampling of hair, faeces, egg shells or feathers (Morin and Woodruff, 1996) can be conducted without the need to capture or disturb individuals of vulnerable species, or observe elusive ones. Faecal surveys, in particular, have been used on a national scale to establish species' ranges (Croose et al., 2013, 2014). At a local scale, changes in the abundance of scats over time have been used to infer relatives change in population density (Harrison et al., 2002; Summers and Denny, 2010). Despite some success in relating faecal abundance to absolute population abundance for species such as sika deer (*Cervus nippon*; Marques et al., 2001) and sambar (*Cervus unicolor*; Brodie, 2006), defining this relationship for has been less successful some species (White tailed deer; *Odocoileus virginianus*; Fuller, 1991, ungulate sp.; Ariefiandy et al., 2013). Variation in diet between individuals or between seasons (Panasci et al., 2011), as well as variable weather conditions or terrain between studies (Murphy et al., 2007; Brinkman et al., 2010), may affect defecation, degradation and detection rates.

Over the last 20 years, advances in molecular techniques have enabled individuals to be identified from non-invasive samples using suites of highly variable microsatellite loci (Morin and Woodruff, 1996; Taberlet and Luikart, 1999). Non-invasive genetic sampling combined with a Spatially Explicit Capture Recapture (SECR) framework (Efford and Fewster, 2013) has proven extremely effective for population estimation of elusive species such as the European wildcat (*Felis silvestris*; Kéry et al., 2010) and jaguar (*Panthera onca*; Tobler et al., 2013). These techniques provide a promising basis for studying European pine marten (*Martes martes*) populations in the UK.

After heavy persecution in the early 20$^{th}$ century, pine marten populations have now recolonized much of their historic range in Scotland (Lockie, 1964; Croose et al., 2013). As a protected native predator, there is strong stakeholder interest in their conservation as well as in their potential role in controlling invasive species such as American grey squirrels (*Sciurus carolinensis*) in the UK (Sheehy and Lawton, 2014). There is also concern, however, about their impact on prey, such as through nest predation of capercaillie (*Tetrao urogallus*) populations (Summers et al., 2009). Knowledge of spatio-temporal changes in population density and how habitat may affect future range expansion may provide important guidance to species conservation policy and practice.

Conservation management based on research conducted in other parts of the pine martens range is, however, likely to be misguided, as strong differences in life history traits exist. In contrast to the specialism for closed canopy forests exhibited by Eastern European populations, those in Western Europe, including Scotland, show more generalist behaviour and incorporate scrub and tussock grassland into their range (Pereboom et al., 2008; Mergey et al., 2011; Caryl et al., 2012). Population density is, therefore, higher in areas with more fragmented forests as the availability of suitable foraging habitat increases. This relationship only holds up to a point, as other factors such as resting and denning sites are lost when the level of fragmentation increases past a

*Corresponding author
*Email address:* l.kubasiewicz@exeter.ac.uk (Laura M. Kubasiewicz)

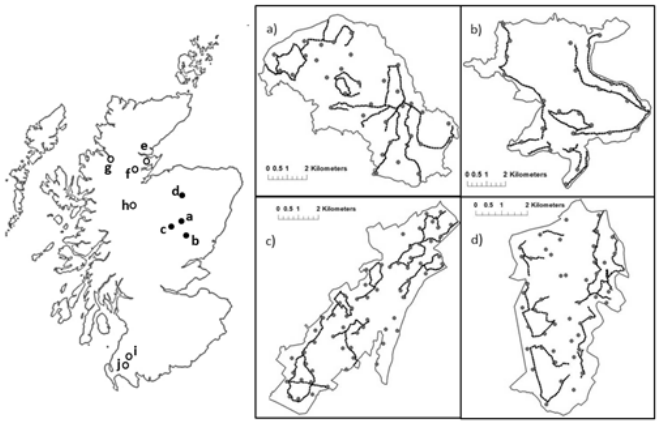


**Figure 1** – Location of the four sites (a - d) used for the collection of hair and scat samples for population density estimates using genetic SECR modelling: a) Abernethy b) Mar c) Inshriach and d) Darnaway. Thin grey lines are site boundaries. thick black lines are scat transects and grey dots are hair tube locations. Data from studies of the remaining sites (e-h) were included to assess the effect of habitat fragmentation on pine marten home range size: e - Morangie (Caryl et al., 2012), f - Novar (Halliwell, 1997), g – Kinlochewe and h - Strathglas (Balharry, 1993), i – Minnoch and j – Glen Trool (Bright and Smithson, 1997)). A colour version of this figure can be viewed in the online version of this article.

threshold level, resulting in a reduction in population density (Birks et al., 2005; Caryl et al., 2012).

Previous non-invasive surveys have taken place to identify pine martens (Mullins et al., 2009) and estimate population density in Ireland (Sheehy, 2013; O'Mahony et al., 2015) and in one forest in Scotland (Croose et al., 2015). The majority of studies in Scotland have employed trapping and radio tracking to estimate population size via the measurement of home ranges (Balharry, 1993; Bright and Smithson, 1997; Halliwell, 1997; Caryl et al., 2012). Here, we used non-invasive genotyping of scat and hair samples, combined with spatially explicit capture recapture models, to provide density estimates of pine marten populations in Scottish forests. We used these data to test a proposed curvilinear relationship between population density and forest fragmentation, suggesting that marten densities reach a peak at moderate levels of forest fragmentation. Finally, we examined whether our population estimation approach can be used to calibrate the traditional method based on scat count.

## Materials and methods

### Study sites

Four sites were surveyed for scat and hair samples from mid-September to mid-November 2011 and mid-September to mid-November 2012 (Fig. 1). Abernethy National Nature Reserve (hereafter Abernethy; 57°15′ N, 3°40′ W) is a Royal Society for the Protection of Birds (RSPB) reserve consisting of Scots pine (*Pinus sylvestris*) plantations and Caledonian pinewood remnants. Mar Lodge Estate (hereafter Mar; 57°0′ N, 3°37′ W) is a Caledonian pinewood owned by the National Trust for Scotland. Inshriach Forest (hereafter Inshriach; 57°6′ N, 3°56′ W) is a Forestry Commission owned Scots pine plantation with some remnants of Caledonian pinewood. Darnaway Forest (hereafter Darnaway; 57°33′ N, 3°45′ W), managed by Moray Development Company Ltd, contains a plantation of Scots pine, Sitka spruce (*Picea sitchensis*) and patches of deciduous woodland. Several other mustelid species are present in the study area, including stoats (*Mustela erminea*), weasels (*Mustela nivalis*), badgers (*Meles meles*), otters (*Lutra lutra*) and mink (*Neovison vison*). Foxes (*Vulpes vulpes*) and red squirrels (*Sciurus vulgaris*) are common throughout the study area but numbers of the former are managed in at least one of the study sites (Abernethy).Sites were selected to provide a range of forest fragmentation levels, and at a sufficient distance apart to reduce the risk of the movement of pine marten between sites.

In addition, data from four previous studies were used for the analysis of the effect of forest fragmentation, providing estimates of home range sizes for six sites within Scotland (Fig. 1). Home ranges were estimated using radio tracking data collected in Morangie Forest from June 2006 to July 2007 (Caryl et al., 2012), Novar Forest from August 1993 to April 1995 (Halliwell, 1997), Kinlochewe for 18 months during 1988 to 1989, Strathglass for 18 months during 1989 to 1991 (Balharry, 1993) and Glen Trool and Minnoch from July 1995 to December 1996 (Bright and Smithson, 1997).

### Sample collection

Scat collection transects (gravel tracks; Fig. 1) were selected from 1:25,000 Ordnance Survey maps and were adjusted or shortened during the first site visit where access was not possible. Transects covered all accessible gravel roads to provide the maximum coverage within each forest, although major access roads were not included unless they provided the shortest route to another gravel track. Transects were cleared of all scats five days prior to sample collection. Sampling sessions were held on three or four consecutive days in each forest, depending on the total transect length (see Tab. 1). Using a fresh toothpick, a portion of each scat was placed into an individually labelled tube for DNA analysis. The remainder of each scat was stored for diet analysis (data not presented).

Plucked hair samples were collected using hair tubes fitted with 1 $cm^2$ sticky pads (Mullins et al., 2009), during Sept-Nov 2011 (Abernethy, Mar) and Sept-Nov 2012 (Darnaway, Inshriach). Hair tube locations were identified using 1:25,000 Ordnance Survey maps, with one (Abernethy Forest, Mar Forest) or two (Inshriach, Darnaway) hair tubes per 1 $km^2$ grid cell within each study area. For ease of access and installation, hair tubes were only placed in grid squares containing both forest tracks and trees. A combination of Hawbakers marten lure (F&T Fur Harvester's Trading Post, MI 49707), peanut butter and bread were used as attractants as these have previously proven effective (Chandrasekhar, 2005; Roche, 2008; Burki et al., 2009).

Sticky pads and attached hair samples were collected in separate polythene bags and labelled with a unique identifier. All samples were frozen at −20 °C within 8hrs and transferred to −80 °C within three weeks to await DNA analysis. Scat and hair collection was carried out simultaneously, with additional hair collection sessions held immediately after scat sampling had been completed for each successive forest (Tab. 1). No hair samples were obtained for Darnaway, excluding this site from further analysis.

### Genetic analysis

To avoid contamination, extractions were performed in a dedicated DNA extraction area. Hair samples were removed from sticky pads with xylene. DNA extractions from scat samples were performed using the QIAamp DNA stool mini kit (Qiagen, #51504). DNA extractions from hair samples were conducted using an adapted Chelex-100 protocol (Walsh et al., 1991); a 1 cm root-section of one to >15 hairs was agitated in 200 µl Chelex (5%), 7 µl dithiothreitol (DDT) and 1µl proteinase at 56 °C for approximately 5 hrs, centrifuged for 3 mins and incubated at 95 °C for 10 mins. One negative control was include in all batches of DNA extraction i.e. one extraction protocol was performed without any hair or faecal matter. One extraction was performed using a pine marten tissue sample (from a road-killed individual) to provide a positive control for amplification and sequencing. Extracted DNA template was stored at −20 °C. DNA template was initially amplified using eight species-specific microsatellite markers (Mar08, Mar21, Mar36, Mar43, Mar53, Mar56, Mar58, Mar64; Natali et al. 2010) in a 10 µl reaction containing 0.4 µmol forward and reverse primers, 5 µl Qiagen Type-it PCR mastermix, 1 µl Q solution and 2 µl DNA template. After initial denaturation at 95 °C for 5 mins, 40 cycles of 95 °C for 30 s, 63 °C for 90 s and 72 °C for 30 s were used followed by a final extension step of 60 °C for 30 mins. Each round of PCR included a positive and negative control from the extraction step. Fragment analysis was performed at DNA Sequencing and Services (University of Dundee, Scotland, DD1 5EH) and included a positive and negative control. After the first plate of samples was sequenced, two samples that amplified successfully at all markers were selected as additional positive controls for

**Table 1** – Details of study sites used for pine marten scat and hair collection during Sept-Nov 2011 and 2012.

| | Abernethy | Mar Forest | Inshriach | Darnaway |
|---|---|---|---|---|
| **Total forested area ($km^2$)** | 39 | 24 | 35 | 24 |
| **Total transect length ($km^2$)** | 106 | 74 | 96 | 80 |
| **No. scat collection sessions** | 3 | 3 | 3 | 3 |
| **No. hair collection sessions** | 4 | 5 | 5 | 4 |
| **No. hair tubes** | 34 | 26 | 64 | 47 |
| **No. Scats** | 239 | 84 | 167 | 92 |
| **No. Hair samples** | 31 | 31 | 51 | 0 |

all subsequent rounds of amplification and sequencing. Samples were scored using GeneMarker (Version 2.4.0) and verified by eye.

Sex typing of hair samples was performed using a 5' nuclease TaqMan assay developed by Mullins et al. (2009) and Real-time PCR using 5 µL Precision Master Mix (Primer Designs), 0.2 mM of either MMX or MMY forward and reverse primers and probes (see Supplement S1) and 3 µL DNA template in a total volume of 10 µL (Mullins et al., 2009). The PCR conditions consisted of 2 mins at 50 °C, 10 mins at 95 °C, then 50 cycles of 15 s at 95 °C and 1 min at 60 °C. Two replicate amplifications were performed for each primer/probe combination. For real-time product detection, Ct value was recorded at a ΔRn threshold of 0.2, with success at Ct $\leqslant$40. A sex type was accepted when both replicate amplifications were successful and matching.

Allelic drop out (ADO), where an allele from the consensus single locus genotype fails to amplify; and false alleles (FA), where an allele not present in the consensus single locus genotype is amplified (Murphy et al., 2007), can cause overestimation of the number of individuals as samples from the same individual appear to differ. Conversely, low genetic diversity can cause the opposite bias, as multiple individuals may have matching genotypes. To reduce the possibility of these erroneous or concealed genotypes, consensus multilocus genotypes were obtained for the initial eight markers following the comparative multi-tubes approach. All eight markers for each sample were initially amplified twice, with up to five subsequent amplifications until each genotype had been observed twice for heterozygous calls and three times for homozygous calls (for a full description see Frantz et al., 2003). For each sample, a genotype was only accepted once a consensus was reached for all eight markers. Samples with sex-types that did not match the consensus result were discarded. One DNA extraction was performed per sample and error rates (ADO and FA) were quantified across all amplification attempts from this template DNA for each locus using calculations provided in Broquet and Petit (2004).

To further strengthen the individual genotype assignments, hair samples with a complete multilocus genotype at eight loci were analysed with a further three microsatellite markers, Mar02 (Natali et al., 2010) Mel1 and Mvi1341 (Sheehy and Lawton, 2014), using the same PCR protocol as for the previous microsatellites. Due to limited DNA stocks, the final three markers were only amplified once. Where the additional three markers failed to amplify, the sample genotype was accepted from the initial eight markers. Furthermore, the probability of sampling more than one individual with the same genotype (Pgen), was calculated for each unique genotype as follows:

$Pgen=(\Pi P_i)2^h$

where $P_i$ is the frequency of each allele (two per locus) in the population represented in the genotype and $h$ is the number of heterozygous loci. See Parks and Werth (1993) for assumptions and caveats. The mean number of alleles, observed heterozygosity (Ho) and expected heterozygosity (He) per locus were calculated in GIMLET version 1.3.3 (Valiere, 2002).

Several other species which may be able to access the hair tubes exist in the study area, such as stoats, weasels and red squirrels. When there was sufficient DNA remaining after the genotyping exercise had been carried out, species-identification was carried out using a universal primer pair based on a region of the mitochondrial cytochrome b D-loop (primers H16498; 5'-CCTGAACTAGGAACCAGATG-3'; Shields and Kocher (1991), and LRCB1; 5'-TGGTCTTGTAAACCAAAAATGG-3'; Davison et al. (2001)). The primers amplified a region of approximately 400 bp which was sequenced and BLASTed against Genbank to confirm that the genotyped hair samples were derived from pine martens. In total 28 unique genotypes were identified across the three sites with 11, 6 and 11 genotypes in Inchriach, Mar Lodge and Abernethy respectively. There was sufficient DNA extract remaining to amplify 18 of the 28 genotypes with the cytochrome b D-loop primers.

## Population assessment

For population density estimation, a SECR model was applied to hair genotypes (Borchers and Efford, 2008) using the `secr` package (v2.8.1; Efford et al., 2004) in R version 3.1.3 (R core team, 2014), with full likelihood estimation for Poisson distribution of home range centres; half normal spatial capture probability distribution; and detection via "proximity detectors" (e.g. camera traps; Borchers, 2012). A buffer was extended beyond the location of traps for each forest. To ensure that this buffer encompassed the effective trapping area, models were tested with increasing buffer widths until a stable density estimate was reached, providing buffer widths of 1 km for Inshriach, 2 km for Abernethy and 3 km for Mar. To determine whether the sex ratio of animals within a forest differs from 1:1, a hybrid mixture model was fitted with fixed class membership (male or female) for individuals of known sex and class-specific detection parameters (g0 and $\sigma$). Deviation from the 1:1 sex-ratio was tested using a likelihood ratio test between the full model and a model with a fixed 1:1 mixing proportion.

Abundance estimates were calculated using `region.N` function in the `secr` package in R (v2.8.1; Efford et al., 2004). As previous analyses on the effect of forest fragmentation used home range size (100% minimum convex polygons) as an index of abundance (Balharry, 1993; Bright and Smithson, 1997; Halliwell, 1997; Caryl et al., 2012), our data were converted to 95% home range sizes for males and females using $\Pi*(2.24*\sigma)^2$ (Efford, unpublished data), where $\sigma$ is the spatial scale of the detection function, or the scale over which an individual is detected, generated from the SECR model for each forest. Conversion of $\sigma$ to the home range radius (i.e. multiplication by 2.24) is determined by the detection function which, in this case, describes a half-normal probability of detection.

## Fragmentation analysis

Estimates of percentage forest cover (%) and forest edge density (m/ha) within a 9.77 km radius of the forest centre (300 $km^2$) were presented as an indices of forest fragmentation in Caryl et al. (2012) for all previous study sites. The same area was used in the current analysis for all additional sites for consistency. Fragmentation indices were calculated for the current study sites using the Land Cover Map (LCM) 2007 land-use layer (Morton et al., 2011). Separate generalised linear models with log link function and Gaussian distribution were used to assess the effect of forest cover and forest edge on home range size for male and female pine martens. A quadratic term for forest cover or edge was included in the starting model and its significance tested using a likelihood ratio test between the starting model and a model with the linear term only. Correlation between the fragmentation indices was tested using `cor.test` in R version 3.1.3.

### Scat density calibration

To test for an association between the number of scats found per km of transect and population density, a generalised linear model with Poisson distribution was performed between scat count values and average home range size for the current sites and from previous studies (Bright and Smithson, 1997; Halliwell, 1997; Caryl et al., 2012). The time between the clearance of transects and subsequent collection of scats in these studies varied from four days to one month (pine marten scats have been observed to exhibit minimal deterioration after one month; Kubasiewicz et al., 2016). Home range estimates were made simultaneously to scat collections in all studies. The number of scats per km was standardised to five days for all studies, assuming equal deposition of scats per day; and only collections that were carried out in autumn were included.

## Results

### Genetic analysis

In total, 579 scats were collected from the four forests. Individual genotyping was attempted for 76 samples, whereby between three and seven replicate amplification attempts were conducted at all eight markers for each sample. A consensus multilocus genotype at all eight loci was gained for 6 samples (7.9%) after 6 amplification attempts, with no additional consensus multilocus genotypes resulting from the seventh amplification attempt. Due to the low level of success of individual genotyping scat samples, further analysis was carried out for hair samples only. A total of 115 hair samples were collected. One locus (mar58) was monomorphic and therefore excluded from further analyses. A consensus multilocus genotype at all seven loci was gained for 83 samples (72%) after a maximum of seven amplification attempts at each loci. Of the 83 samples that provided a consensus genotype, 28 individual pine marten were identified, with a total of 55 recaptures. This equates to 11 unique genotypes for Abernethy, six for Mar and 11 for Inshriach. The maximum number times one individual was recaptured was 11, whilst 13 pine martens were only captured once.

Overall, 6% of the successful amplifications experienced allelic drop out and 7% contained false alleles. Per locus, allelic dropout (0–11%) occurs less frequently than false alleles (2–14%; Tab. 2). For the additional three markers used latterly in the study, a single locus genotype was reached for 68% of amplifications (59 samples, one amplification attempt per locus per sample). The addition of these loci did not further divide any existing individual genotypes. Nine pairs of individuals differ at only one locus, with six pairs containing an individual that only occurred once in the samples (Table S2). Sex-types were obtained for seven individuals from Abernethy (63%), five from Mar (83%) and 11 from Inshriach (100%).

An average of 2.9 alleles per locus was found across all samples. Overall, observed heterozygosity (Ho) was 0.55 and expected heterozygosity (He) was 0.4. Significant deviation from Hardy-Weinberg equilibrium was observed at two loci; mar53 ($\chi^2$=23.9, df=3, $p$<0.001) and mar64 ($\chi^2$=62.8, df=6, $p$<0.001). A consensus multilocus genotype at all seven initial loci was obtained for 82 samples (71%), with 35 of these also supported by all three additional markers (30%), the remaining 47 samples either did not amplify with the three additional loci or did not have sufficient extract left to attempt further amplifications. Genotype probabilities (Pgen) for individuals ranged from $1.90\times10^{-5}$ to $4.14\times10^{-2}$ (Table S2); average genotype probabilities for forests were $4.01\times10^{-4}$ for Abernethy, $2.95\times10^{-4}$ for Inshriach and $9.63\times10^{-3}$ for Mar.

The 18 DNA genotypes that were amplified using mitochondrial D-loop primer pair were all confirmed as having been derived from pine marten. A single haplotype was recorded which had a 100% match to Martes martes haplotype Mm28 (GenBank accession HM026017.1). We were unable to test the remaining ten genotypes due to lack of remaining extract but the fact that these samples amplified using the microsatellite primer pairs developed specifically for European pine marten supports our contention that these genotypes were also those of pine marten. In addition, when we tested hairs from other tree dwelling mammals such as red squirrel, the samples failed to amplify using our panel of microsatellite primers. This is support that the panel of microsatellites is specific in its amplification of pine marten DNA.

### Density estimates

Population density estimates using SECR models were 0.38 individuals km$^{-2}$ (95% CI 0.11–1.07) for Abernethy, 0.07 individuals km$^{-2}$ (0.03–0.16) for Mar and 0.24 individuals km$^{-2}$ (0.13–0.45) for Inshriach. Population estimates were 17 (95% CI 12–47) for Abernethy,

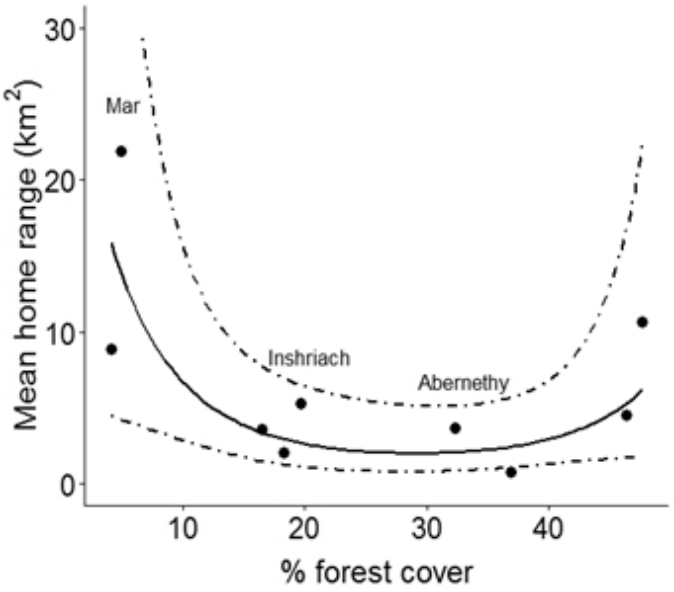


**Figure 2** – Average home range size of female pine marten with decreasing levels of forest fragmentation (% forest cover). Data points are for observed data, solid lines represent predicted female home range size from the GLMM and dashed lines represent the 95% confidence intervals for the model prediction calculated from 1000 repeated model simulations using random draws from the estimated parameter distributions. Data from the current study (labelled) were estimated as 100% minimum convex polygons and added to dataset from Balharry (1993); Halliwell (1997); Bright and Smithson (1997); Caryl (2008), which provided 95% minimum convex polygon home ranges.

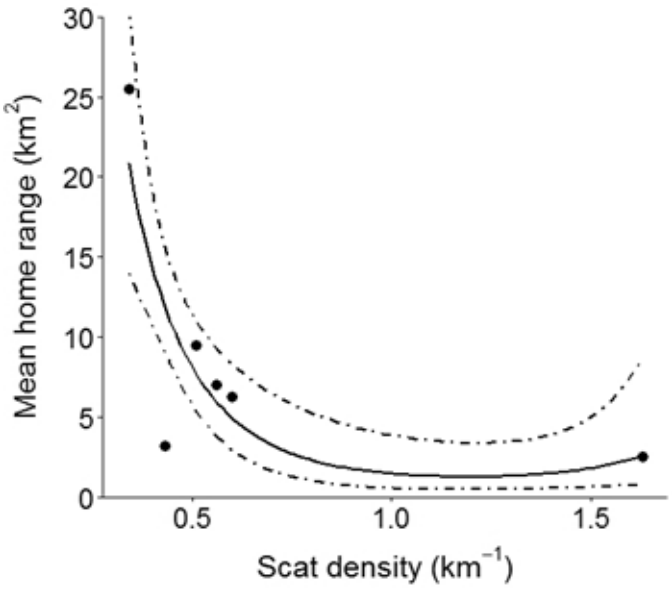


**Figure 3** – Average pine marten home range size with increasing scat density (scats km$^{-1}$). Data points are for observed data, solid lines represent predicted home range size from the GLM and dashed lines represent the 95% confidence intervals for the model prediction calculated from repeated model simulations using random draws from the estimated parameter distributions. Data were taken from the current study, Halliwell (1997), Bright and Smithson (1997) and Caryl (2008). Where home ranges were provided per sex, a mean was calculated. Scat density was standardised to give values for day 5 between clearance and collection..

**Table 2** – Rates of allelic drop out (ADO) and false alleles (FA); expected (exp) and observed (obs) heterozygosity; and number of alleles found for each marker for all hair samples collected from Abernethy Forest, Mar Forest and Inshriach forests combined.

| Marker | ADO (%) | FA (%) | H (exp) | H (obs) | No. alleles |
|---|---|---|---|---|---|
| m08 | 11 | 2 | 0.63 | 0.60 | 5 |
| m43 | 0 | 8 | 0.02 | 0.02 | 2 |
| m56 | 8 | 15 | 0.51 | 0.80 | 5 |
| m21 | 2 | 7 | 0.50 | 0.57 | 2 |
| m53 | 6 | 4 | 0.60 | 0.72 | 3 |
| m64 | 4 | 4 | 0.66 | 0.91 | 4 |
| m36 | 0 | 4 | 0.01 | 0.01 | 2 |
| m02 | - | - | 0.47 | 0.60 | 2 |
| mel01 | - | - | 0.47 | 0.77 | 2 |
| Mvi1341 | - | - | 0.38 | 0.50 | 2 |
| **Mean (all markers)** | 6 | 7 | 0.42 | 0.55 | 2.9 |

6 (95% CI 6–11) for Mar and 11 (95% CI 11–24) for Inshriach Tab. 3). The proportion of males in the population did not differ significantly from 0.5 for Mar (proportion males=0.43; $\chi^2$=7.11, df=3, $p$=0.068) or Inshriach (proportion males=0.45; $\chi^2$=0.07, df=1, $p$=0.792), but was 0.2 in Abernethy ($\chi^2$=4.596, df=1, $p$=0.032).

### Fragmentation analysis

The level of forest fragmentation ranged from 4.9% forest cover and 145.7 m ha$^{-1}$ forest edge (Mar) to 32.3% forest cover and 86.6m ha$^{-1}$ forest edge (Abernethy). Including data from previous analyses, a strong negative correlation was found between forest edge and percentage forest cover (Pearson correlation r=-0.856, df=7, $p$=0.003). Results are therefore presented for the analysis between home range size and percentage forest cover only. A highly significant non-linear relationship was found between percentage forest cover and female home range size (Fig. 2, Tab. 4); the GLM predicted a decrease in home range size from 16 km$^2$ (95% CI: 4–55) to 4 km$^2$ (95% CI: 2–8) as forest cover increases from 4% to 20%, followed by an increase in home range size to 7 km$^2$ (2–22) as forest cover increases to 48%. This increase is, however, strongly influenced by a single data point (Minnoch Forest). The relationship between male home ranges and forest cover was non-significant ($\chi^2$=1.60, df=1, $p$=0.08).

### Scat count calibration

A significant negative non-linear relationship was found between scat count and home range size ($\chi^2$=10.96, df=1, $p$=0.005; Fig. 3). As scat count increased from 0.1 to 0.5 scats km$^{-1}$, there was a substantial decline in home range from 21 km$^2$(14–32 km$^2$) to 4 km$^2$(2–6 km$^2$), followed by a much more gradual decline to 3 km$^2$(1–8 km$^2$) as scat count reached 1.5 scats km$^{-1}$.

## Discussion

Here we provide the first population density estimates for pine marten populations in Scotland obtained using a combination of non-invasive multilocus genotyping of hair samples and SECR modelling, providing a valuable baseline to support more sophisticated monitoring and refined management decisions.

The pine martens from the Scottish populations sampled here have relatively low allelic diversity (mean alleles per locus=2.9) and heterozygosity (mean alleles per locus=5.5 and 7.25, mean Ho=0.64 and 0.713, mean He=0.67 and 0.77; Mullins et al., 2009; Bartolommei et al., 2016). The relatively recent population bottleneck experienced by Scottish pine marten may have had lasting impacts on the genetic diversity of the species, although a larger scale study incorporating more of their range in Scotland is needed to confirm this.

It is possible that some individuals within the study sites were averse to hair tubes which would lower the probability of capture and may lead to under-estimates of population size. The inclusion of individual genotypes from scat samples would have reduced this risk had the genetic results for scats been more successful (i.e. as in Croose et al., 2015). However, despite relatively low genetic diversity, the probability of matching genotypes between individuals (Pgen) was low and the inclusion of three extra markers did not further sub-divide the data into more individuals, suggesting that we did not under-estimate the size of the population (Parks and Werth, 1993). It is possible, however, that we may have slightly over estimated the population densities in these forests. Firstly, genotyping errors were slightly higher than for previous studies using DNA extracted from hair (Mullins et al., 2009; Baldwin et al., 2010; Fickel et al., 2012; Uno et al., 2012), which may mean we classified samples from the same individual as being from different individuals. This is most likely to have occurred within one of the nine pairs of individuals that differed at a single locus. Furthermore, home ranges from previous data are based on mean 100% minimum convex polygons, whereas those from current data are 95% estimates. Currently, there is no tested formula to convert these estimates to 100% home ranges (Murray Efford, pers. comm.). Our home range sizes may therefore be under-estimates (the equivalent of over-estimating population density) relative to those from previous studies included in the dataset.

The current results provide considerable support for previous findings that pine martens in Scotland are less forest dependent than the populations in Eastern Europe (Pereboom et al., 2008; Mortelliti et al., 2010; Caryl et al., 2012). We provide a clearer picture of the relationship between forest cover and pine marten population density; the highest population densities are found with forest cover of between 20% and 35%, likely due to a balance between the availability of forest resources such as den sites (Birks et al., 2005) and the foraging opportunities provided by non-forested habitats (Caryl et al., 2012). Furthermore, we suggest that this trend is attributed to female pine martens, which are known to select their territory based on the availability of suitable resources for rearing young, such as den sites (Powell, 1994), whilst males locate their territory with respect to the location of females (Caryl, 2008). As the decrease in population size predicted at higher levels of forest cover is strongly dependent on one data point, additional data at this end of the scale would be a valuable addition for subsequent analyses. This finding raises the question of how to incorporate the conservation of this species with other management goals for the forested landscape, particularly in light of the dynamic nature of land use in Scotland and ongoing debate regarding the extent and placement of woodland (Sing et al., 2013). Population density varies according to other environmental factors not tested in the current analysis. For example, pine marten abundance is negatively associated with increasing proportions of agriculture in Finland (Kurki et al. 1998) and this habitat type is avoided by pine martens in Scotland (Caryl et al., 2012). The forests in the current study are also located throughout Scotland, with two sites in Dumfries and Galloway where a higher proportion of forest is intensively managed plantation forest woodland which is avoided by male pine martens at both a landscape and local level (Caryl et al., 2012). Forest composition, as well as fragmentation, should therefore be considered when developing plans for management.

**Table 3** – Population density and abundance estimates (km$^{-2}$) for pine marten in Abernethy, Mar Forest and Inshriach forests. Sex-specific detection parameter estimates (g0, sigma, pmix), where pmix represents the proportion of the population belonging to each sex. Minimum population numbers (Min. pop.) represent the number of unique genotypes detected in each forest, and so the minimum population present. The population estimate (Pop. est.), standard error and confidence intervals are the model results from the `region.N` function in the SECR program in R. Home range estimates were calculated using the formula $\Pi^*(2.24^*\sigma)^2$ (Efford; unpublished data), using the mean value of sigma within each forest between males and females.

| | Parameter | Estimate | SE | LCL | UCL | Min. pop. | Pop. est. | SE | LCL | UCL | Home range (km$^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Abernethy** | Density | 0.384 | 0.217 | 0.113 | 1.076 | 11 | 17 | 6.9 | 12 | 47 | 7.0 |
| Female | g0 | 0.156 | 0.125 | 0.028 | 0.543 | | | | | | |
| | sigma | 480 | 185 | 232 | 995 | | | | | | 3.63 |
| | pmix | 0.849 | 0.106 | 0.523 | 0.967 | | | | | | |
| Male | g0 | 0.317 | 0.129 | 0.126 | 0.600 | | | | | | |
| | sigma | 856 | 153 | 605 | 1212 | | | | | | 11.6 |
| | pmix | 0.151 | 0.107 | 0.033 | 0.477 | | | | | | |
| **Mar Forest** | Density | 0.065 | 0.029 | 0.028 | 0.148 | 6 | 6 | 0.8 | 6 | 11 | 18.8 |
| Female | g0 | 0.368 | 0.129 | 0.164 | 0.633 | | | | | | |
| | sigma | 1179 | 230 | 808 | 1721 | | | | | | 21.9 |
| | pmix | 0.535 | 0.236 | 0.153 | 0.881 | | | | | | |
| Male | g0 | 0.316 | 0.153 | 0.104 | 0.649 | | | | | | |
| | sigma | 1006 | 203 | 679 | 1489 | | | | | | 15.9 |
| | pmix | 0.464 | 0.236 | 0.119 | 0.847 | | | | | | |
| **Inshriach** | Density | 0.241 | 0.079 | 0.129 | 0.449 | 11 | 11 | 1.9 | 11 | 24 | 11.7 |
| Female | g0 | 0.340 | 0.096 | 0.178 | 0.551 | | | | | | |
| | sigma | 576 | 81 | 436 | 759 | | | | | | 5.23 |
| | pmix | 0.499 | 0.159 | 0.222 | 0.776 | | | | | | |
| Male | g0 | 0.413 | 0.023 | 0.013 | 0.120 | | | | | | |
| | sigma | 1125 | 349 | 621 | 204 | | | | | | 19.9 |
| | pmix | 0.501 | 0.159 | 0.224 | 0.778 | | | | | | |

Home range sizes are smaller for female pine martens compared to males (Balharry, 1993; Caryl et al., 2012). The ratio of males to females within a population, however, has not previously been examined. Although the proportion of forest and non-forest habitat within an area is an important determinant of habitat suitability, non-forested areas support different ecological functions, such as foraging or resting (Dunford and Freemark, 2005; Caryl et al., 2012). The high proportion of females at Abernethy may, therefore, be due to the composition of non-forest habitat within the site, although additional data from other forests with a similar habitat composition to Abernethy would be useful to confirm this.

The calibration of the scat count method provides the first evidence that pine marten density could be inferred from scat abundance, although we urge caution in doing so based on current information. We found a negative non-linear relationship between home range size and scat counts, indicating a positive effect of population density on scat abundance but only at relatively low densities. However, the sample size used in the analysis was very small, and the trend is strongly influenced by two data points at either end of the scale. Several factors result in an uncertain relationship between scat abundance and population density, although some of these can be controlled for by careful sampling and standardised procedures. For instance, defecation rate can vary depending on diet (Panasci et al., 2011) and degradation rate can depend on weather (Murphy et al., 2007; Brinkman et al., 2010). Detection errors may also occur if faeces from other species are misidentified as pine marten or vice versa (Davison et al., 2002; Birks et al., 2004), or there is reduced visibility of scats where paths are overgrown, uneven or heavily used by vehicles (McHenry et al., 2016). Unfortunately, limited data prevented a more detailed analysis, although future modelling attempts may provide an invaluable monitoring tool for this species.

**Table 4** – Coefficient estimates for the GLM for the effect of forest cover on female pine marten home range size. Estimates are for the full model. Log-likelihood $\chi^2$ statistic and associated p-values are for the deletion of Cover$^2$ from the full model; or Cover from the model with main effects only.

| | Estimate ± SE | $\chi^2$ | df | *p* |
|---|---|---|---|---|
| **Intercept** | 3.505 ± 0.739 | | | |
| **Cover** | -0.194 ± 0.069 | 7.027 | 1 | 0.206 |
| **Cover$^2$** | 0.003 ± 0.001 | 3.313 | 1 | 0.009 |

As pine marten continue to reclaim their former range in Scotland, close monitoring may reveal further differences between the behaviour exhibited by Scottish populations and those elsewhere in Europe. As a native mammalian predator, one of few remaining in Britain, conservation management should seek to attain a balance between enabling pine marten to continue recovery of their former range with the need to protect vulnerable prey species. This will require detailed knowledge of their distribution and population density across a range of habitats. Here we provide evidence that non-invasive hair samples can enable the cost-effective, large scale monitoring that would enable this balance to be achieved.

## References


Ariefiandy A., Purwandana D., Coulson G., Forsyth D.M., Jessop T.S., 2013. Monitoring the ungulate prey of the Komodo dragon *Varanus komodoensis*: distance sampling or faecal counts? Wildlife Biol. 19: 126–137.

Baldwin H.J., Hoggard S.J., Snoyman S.T., Stow A.J., Brown C., 2010. Non-invasive genetic sampling of faecal material and hair from the grey-headed flying-fox (*Pteropus poliocephalus*). Aust. Mammal. 32(1): 56–61.

Balharry D., 1993. Factors affecting the distribution and population density of pine martens (*Martes martes* L.) in scotland. PhD Thesis, University of Aberdeen.

Bartolommei P., Gasperini S., Manzo E., Natali C., Ciofi C., Cozzolino R., 2016. Genetic relatedness affects socio-spatial organization in a solitary carnivore, the European pine marten. Hystrix 27(2):222-224. doi:10.4404/hystrix-27.2-11876

Birks J.D.S., Messenger J., Braithwaite T., Davison A., Brookes R., Strachan C., 2004. Are scat surveys a reliable method for assessing distribution and population status of pine martens? In: Harrison D.J., Fuller A.K., Proulx G. (Eds.) Martens and Fishers (Martes) in Human-altered Environments. New York, Springer-Verlag. 235–252.

Birks J.D.S., Messenger J.E., Halliwell E.C., 2005. Diversity of den sites used by pine martens *Martes martes*: a response to the scarcity of arboreal cavities? Mammal Rev. 35: 313–320.

Borchers D., 2012. A non-technical overview of spatially explicit capture-recapture models. J. Ornithol. 152: S435–S444.

Borchers D.L., Efford M.G., 2008. Spatially explicit maximum likelihood methods for capture-recapture studies. Biometrics 64(2): 377–385.

Bright P.W., Smithson T.J., 1997. Species recovery programme for the pine marten in England: 1995–96. English Nature Research Reports 240: 1–64.

Brinkman T.J., Person D.K., Schwartz M.K., Pilgrim K.L., Colson K.E., Hundertmark K.J., 2010. Individual identification of Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) using DNA from fecal pellets. Conservation Genet. Resour. 2: 115–118.

Brodie J.F., 2006. An experimentally determined persistence-rate correction factor for scat-based abundance indices. Wildl. Soc. Bull. 34: 1216–1219.

Broquet T., Petit E., 2004. Quantifying genotyping errors in noninvasive population genetics. Mol. Ecol. 13(11): 3601–3608.

Brøseth H., Nilsen E.B., Pedersen H.C., 2012. Temporal quota corrections based on timing of harvest in a small game species. Eur. J. Wildl. Res. 58(5): 797–802.
Burki S., Roth T., Robin K., Weber D., 2009. Lure sticks as a method to detect pine martens *Martes Martes*. Acta Theriol. 55(3): 223–230.
Caryl F., 2008. Pine marten diet and habitat use within a managed coniferous forest. PhD Thesis, School of Biological & Environmental Sciences, University of Stirling.
Caryl F.M., Quine C.P., Park K.J., 2012. Martens in the matrix: the importance of nonforested habitats for forest carnivores in fragmented landscapes. J. Mammal. 93(2): 464-474.
Chandrasekhar A., 2005. The evaluation of bait-marking as a method for delineating pine marten (*Martes martes*) territories in South-east Ireland. MSc Conservation Biology, University of Kent.
Croose E., Birks J.D.S., O'Reilly C., Turner P., Martin J., MacLeod E.T., 2015. Sample diversity adds value to non-invasive genetic assessment of a pine marten (*Martes martes*) population in Galloway Forest, southwest Scotland. Mamm. Res. 61: 131–139.
Croose E., BirksJ.D.S., Schofield H.W., 2013. Expansion zone survey of pine marten (Martes martes) distribution in Scotland. Scottish Natural Heritage Commissioned Report No. 520.
Croose E., BirksJ.D.S., Schofield H.W., O'Reilly C., 2014. Distribution of the pine marten (*Martes martes*) in southern Scotland in 2013. Scottish Natural Heritage Commissioned Report No. 740.
Davison A., Birks J.D., Brookes R.C., Messenger J.E., Griffiths H.I., 2001. Mitochondrial phylogeography and population history of pine martens *Martes martes* compared with polecats *Mustela putorius*. Mol. Ecol. 10(10): 2479–2488.
Davison A., Birks J.D.S., Brookes R.C., Braithwaite T.C., Messenger J.E., 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. J. Zool. 257: 141–143.
Dunford W., Freemark K., 2005. Matrix Matters: Effects of Surrounding Land Uses on Forest Birds Near Ottawa, Canada. Landsc. Ecol. 20(5): 497–511.
Efford M.G., Dawson D.K., Robbins C.S., 2004. DENSITY: software for analysing capture-recapture data from passive detector arrays. Anim. Biodivers. Conserv. 27(1): 217–228.
Efford M.G., Fewster R.M., 2013. Estimating population size by spatially explicit capture-recapture. Oikos 122: 918–928.
Fickel J., Bubliy O.A., Brand J., Mayer K., Heurich M., 2012. Low genotyping error rates in non-invasively collected samples from roe deer of the Bavarian Forest National Park. Mamm. Biol. 77(1): 67–70.
Frantz A.C., Pope L.C., Carpenter P.J., Roper T.J., Wilson G.J., Delahay R.J., Burke T., 2003. Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Mol. Ecol. 12(6): 1649–1661.
Fuller T.K., 1991. Do pellet counts index white-tailed deer numbers and population change? J. Wildl. Manage. 55: 393–396.
Halliwell E.C., 1997. The Ecology of Red Squirrels in Scotland in relation to Pine Marten Predation. PhD Thesis, University of Aberdeen.
Harrison R.L., Barr D.J., Dragoo J.W., 2002. A Comparison of Population Survey Techniques for Swift Foxes (*Vulpes velox*) in New Mexico. Am. Midl. Nat. 148(2): 320–337.
Kéry M., Gardner B., Stoeckle T., Weber D., Royle J.A., 2010. Use of Spatial Capture-Recapture Modeling and DNA Data to Estimate Densities of Elusive Animals. Conserv. Biol. 25(2): 356–364.
Kubasiewicz L.M., Minderman J., Woodall L.C., Quine C.P., Coope R., Park K.J., 2016. Fur and faeces: an experimental assessment of non-invasive DNA sampling for the European pine marten. Mammal Research 61(4): 299–307.
Kurki S., Nikula A., Helle P., Linden H., 1998. Abundances of Red Fox and Pine Marten in Relation to the Composition of Boreal Forest Landscapes. J. Anim. Ecol. 67(6): 874–886.
Lockie J.D., 1964. Distribution and Fluctuations of the Pine Marten, *Martes martes* (L.), in Scotland. J. Anim. Ecol. 33(2): 349.
Marques F.F.C., Buckland S.T., Goffin D., Dixon C.E., Borchers D.L., Mayle B.A., Peace A.J., 2001. Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. J. Appl. Ecol. 38(2): 349–363.
McHenry E., O'Reilly C., Sheerin E., Kortland K., Lambin X., 2016. Strong inference from transect sign surveys: combining spatial autocorrelation and misclassification occupancy models to quantify the detectability of a recovering carnivore. Wildl. Biol. 22(5): 209–216.
Mergey M., Helder R., Roeder J.J., 2011. Effect of forest fragmentation on space-use patterns in the European pine marten (*Martes martes*). J. Mammal. 92: 328–335.
Morin P.A., Woodruff D.S., 1996. Non-invasive genotyping for vertebrate conservation. In: Smith T.B., Wayne R.K. (Eds.) Molecular Genetic Approaches in Conservation, Oxford University Press. 298–313.
Mortelliti A., Amori G., Capizzi D., Rondinini C., Boitani L., 2010. Experimental design and taxonomic scope of fragmentation studies on European mammals: current status and future priorities. Mammal Rev. 40(2): 125–154.
Morton D., Rowland C., Wood C., Meek L., Marston C., Smith G., Wadsworth R., Simpson I.C., 2011. Final Report for LCM2007 — the new UK Land Cover Map. CS Technical Report No 11/07, Centre for Ecology & Hydrology (Natural Environment Research Council).
Mullins J., Statham M.J., Roche T., Turner P.D., O'Reilly C., 2009. Remotely plucked hair genotyping: a reliable and non-invasive method for censusing pine marten (*Martes martes*, L. 1758) populations. Eur. J. Wildl. Res. 56(3): 443–453.
Murphy M.A., Kendall K.C., Robinson A., Waits L.P., 2007. The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. Conserv. Genet. 8(5): 1219–1224.
Natali C., Banchi E., Ciofi C., Manzo E., Bartolommei P., Cozzolino R., 2010. Characterization of 13 polymorphic microsatellite loci in the European pine marten *Martes martes*. Conserv. Genet. Resour. 2(S1): 397–399.
O'Mahony D.T., Turner P., O'Reilly C., 2015. Pine marten (*Martes martes*) abundance in an insular mountainous region using non-invasive techniques. Eur. J. Wildl. Res. 61(1): 103–110.
Panasci M., Ballard W.B., Breck S., Rodriguez D., Densmore L.D., Wester D.B., Baker R.J., 2011. Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. J. Wildl. Manage. 75(7): 1616–1624.
Parks J.C., Werth C.R., 1993. A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). Am. J. Bot. 80(5): 537–54
Pereboom V., Mergey M., Villerette N., Helder R., Gerard J.-F., Lodé Th., 2008. Movement patterns, habitat selection, and corridor use of a typical woodland-dweller species, the European pine marten (*Martes martes*) in fragmented landscape. Can. J. Zool. 86: 983–991.
Powell R.A., 1994. Structure and spacing of Martes populations. In: Buskirk S.W., Harestad A., Raphael M., Powell R.A. (Eds.) Martens, sables, and fishers: biology and conservation.Ithaca, New York, Cornell University Press. 101–121.
Remis M.J., Kpanou J.B., 2011. Primate and ungulate abundance in response to multi-use zoning and human extractive activities in a Central African Reserve. Afr. J. Ecol. 49(1): 70–80.
Roche T., 2008. The use of baited hair traps and genetic analysis to determore the presence of Pine marten. MSc thesis, Waterford Institute of technology.
Sheehy E., 2013. The role of the pine marten in Irish squirrel population dynamics. PhD Thesis, National University of Ireland, Galway.
Sheehy E., Lawton C., 2014. Population crash in an invasive species following the recovery of a native predator: the case of the American grey squirrel and the European pine marten in Ireland. Biodivers. Conserv. 23(3): 753–774.
Shields G.F., Kocher T.D., 1991. Phylogenetic Relationships of North American Ursids Based on Analysis of Mitochondrial DNA. Evolution 45(1): 218–221.
Sing L., Towers W., Ellis J., 2013. Woodland expansion in Scotland: an assessment of the opportunities and constraints using GIS. Scott. For. 67: 18–25.
Summers R., Denny R., 2010. The abundance and distribution of pine martens and red foxes at Abernethy Forest and Craigmore Wood in 2009/10, as determined by scat counts. Internal RSPB report, Royal Society for the Protection of Birds.
Summers R.W., Willi J., Selvidge J., 2009. Capercaillie *Tetrao urogallus* Nest Loss and Attendance at Abernethy Forest, Scotland. Wildl. Biol. 15(3): 319–327.
Taberlet P., Luikart G., 1999. Non-invasive genetic sampling and individual identification. Biol. J. Linnean Soc. (68): 41–55.
Tobler M.W., Carrillo-Percastegui S.E., Hartley A.Z., Powell G.V.N., 2013. High jaguar densities and large population sizes in the core habitat of the southwestern Amazon. Biol. Conserv. 159: 375–381.
Uno R., Kondo M., Yuasa T., Yamauchi K., Tsuruga H., Tamate H.B., Yoneda M., 2012. Assessment of genotyping accuracy in a non-invasive DNA-based population survey of Asiatic black bears (*Ursus thibetanus*): lessons from a large-scale pilot study in Iwate prefecture, northern Japan. Popul. Ecol. 54(4): 509–519.
Valiere N., 2002. GIMLET: a computer program for analysing genetic individual identification data. Mol. Ecol. Notes 2(3): 377–379.
Walsh P.S., Metzger D.A., Higuchi R., 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10(4): 506–513.

*Associate Editor:* L.A. Wauters

## Supplemental information

Additional Supplemental Information may be found in the online version of this article:

**Supplement S1** MMX and MMY probe sequences.

**Table S2** Consensus genotypes and sex types of individuals samples within Abernethy NNR, Mar Lodge Estate and Inshriach Forest during Autumn 2011 and 2012.