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Short Communication

Low genotyping error rates in non-invasively collected samples from roe deer of the Bavarian Forest National Park

Joerns Fickel [a,*], Oleg A. Bubliy [a], Julia Brand [a], Kathrin Mayer [b], Marco Heurich [b]

[a] Leibniz-Institute for Zoo and Wildlife Research, Dept. Evolutionary Genetics, Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany
[b] Bavarian Forest National Park, Dept. Research and Documentation, Freyunger Str. 2, D-94481 Grafenau, Germany

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ABSTRACT

Genetic wildlife monitoring is increasingly carried out on the basis of non-invasively collected samples, whereby the most commonly used DNA sources are skin appendages (hairs, feathers) and faeces. In order to guide decisions regarding future adequate ways to monitor the roe deer (Capreolus capreolus) population of the Bavarian Forest National Park in Germany, we tested these two different types of DNA source materials to compare their suitability for genetic monitoring. We determined the haplotypes (d-loop) of 19 roe deer and genotyped each individual (tissue, hairs, faeces) across 12 microsatellite loci. The amount of missing and erroneous microsatellite alleles obtained from hair and faeces samples, respectively, was estimated based on comparisons with the corresponding tissue sample control. We observed no missing alleles in hair samples, but in fecal samples PCR failed in 30 out of 228 instances (19 individuals x 12 loci), corresponding to a frequency of missing alleles of 13.2% across all loci and individuals. In genotypes generated from hairs erroneous alleles were detected in 2 out of 228 instances (0.9%), while genotypes retrieved from fecal samples displayed erroneous alleles in 6 out of 198 remaining instances (3%). We conclude that both hair and fecal samples are generally well suited for genetic roe deer monitoring, but that fecal sample based analyses require a larger sample size to account for higher PCR failure rates.

During the last decades monitoring wildlife genetically has increasingly been carried out by use of non-invasively collected samples (Constable et al. 1995; reviewed in Beja-Pereira et al. 2009). The method allows to even monitor species and populations that are either scarce, elusive, inaccessible, or difficult to count (Davison et al. 2002; Creel et al. 2003; Fickel and Hohmann 2006). In national parks and other protected areas where management requires sustainable populations and preservation of genetic variability on one hand (Fickel et al. 2007) but least disturbances for these populations on the other, this method is particularly valuable as a tool to monitor the success of wildlife population management measures.

Among the larger wildlife in Central Europe, the European roe deer (*Capreolus capreolus*) is a very important game species with more than one million animals shot annually in Germany alone (Wotschikowsky 2010). Due to its feeding strategy (Fickel et al. 1998; Clauss et al. 2003; Sage et al. 2004) it has also a great impact on forest management and is often considered detrimental to forest restoration (Partl et al. 2002). However, an obstacle for proper roe deer management in general and in protected areas in particular is the impossibility to perform accurate roe deer counts (Strandgaard 1972; Pielowski 1984; Gaillard 1988). Therefore, development and implementation of methods allowing better roe deer population size estimates is of high interest. In order to guide decisions regarding future adequate ways to monitor the roe deer population of the Bavarian Forest National Park in Germany, we tested two different types of DNA source material (faeces and hair) to compare their suitability for genetic monitoring.

Roe deer ($n = 21$) were hunted in fall 2010 in the Bavarian Forest National Park and its surrounding forests and DNA was isolated from muscle tissue, plucked hairs and faeces (removed from the anal orifice). Two roe deer did not provide fecal samples and were excluded from subsequent analyses. DNA from hair roots and tissue was extracted using the DNeasy kit (Qiagen, Hilden, Germany) and DNA from faeces was isolated using cotton swabs following Kalz et al. (2006). DNA concentrations were measured spectrophotometrically using a NanoDrop ND1000 (PeqLab GmbH, Erlangen, Germany).

For mitochondrial DNA we sequenced 480 bp of the mtDNA control region using primers CaprCTR-f (5′-CACCACCAACACCCAAAGCT-3′) and CaprCTR-r (5′-CCTGAAGTAAGAACCA GATG-3′) (Wiehler and Tiedemann 1998).

* Corresponding author. Tel.: +49 0 30 5168314; fax: +49 0 30 5126104.
E-mail address: Fickel@IZW-Berlin.de (J. Fickel).

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Table 1
Number of alleles per locus (N_A), allele size range, observed and expected heterozygosities (H_O, H_E) and frequency of null-alleles (f_{null}), estimated using the tissue sample (control).

Locus	N_A	Size range [bp]	H_O	H_E	f_{null}
BM4107[a]	2	154–156	0.316	0.273	0
Haut14[b]	7	123–139	0.474	0.674	0.097
NVHRT16[c]	7	156–176	0.842	0.745	0
NVHRT21[c]	9	146–180	0.895	0.826	0
NVHRT24[c]	5	126–144	0.526	0.686	0.061
NVHRT30[c]	6	152–166	0.526	0.714	0.097
NVHRT48[c]	5	86–94	0.579	0.552	0
Roe01[d]	2	130–132	0.579	0.422	0
Roe05[d]	5	123–131	0.526	0.660	0.035
Roe06[d]	7	94–116	1.000	0.836	0
Roe08[d]	10	65–89	0.789	0.794	0.021
Roe09[d]	3	174–178	0.526	0.472	0

Loci derived from [a,b] cattle (Bishop et al. 1994; Thieven et al. 1995), [c] reindeer (Røed and Midthjell 1998), and [d] roe deer (Fickel and Reinsch 2000).

Table 2
Variation of gene diversity statistics across 12 loci for three types of DNA source material.

Statistics	Material	Range	Mean	CI_{95}
N_A	Tissue	2–10	5.67	4.06–7.28
	Hair	2–10	5.67	4.06–7.28
	Faeces	2–9	5.08	3.61–6.55
H_O	Tissue	0.316–1	0.632	0.503–0.760
	Hair	0.316–1	0.627	0.502–0.753
	Faeces	0.222–1	0.599	0.446–0.752
H_E	Tissue	0.273–0.836	0.638	0.527–0.749
	Hair	0.273–0.836	0.640	0.528–0.752
	faeces	0.203–0.831	0.608	0.494–0.721
f_{null}	Tissue	0–0.097	0.026	0.002–0.050
	Hair	0–0.097	0.026	0.002–0.050
	Faeces	0–0.195	0.046	−0.001–0.093

CI_{95} – 95% confidence interval. N_A – number of alleles, H_O – observed heterozygosity, H_E – expected heterozygosity at HWE, f_{null} – null-allele frequency.

The PCR mixture (15 μl) contained 0.1 μl of FastStart polymerase (5U/μl; Roche Diagnostics GmbH, Mannheim, Germany), 1.5 μl 10× FastStart reaction buffer (containing 20 mM $MgCl_2$; Roche, Mannheim Germany), 1.2 μl dNTPs (10 mM), 0.6 μl of each primer (10 μM), 1 μl BSA (2 μg/μl), 3 μl DNA (50–120 ng), 7 μl H_2O. Cycling conditions were 95 °C 5 min, 35× {95 °C 30 s, 50 °C 30 s, 72 °C 30 s}, 72 °C 7 min on a PeqStar PCR instrument (PeqLab). Fragments were purified using ExoSAP-IT (GE Healthcare, Munich, Germany), sequenced bidirectionally (BigDye™ cycle sequencing kit v.1.1) using either one of the above mentioned primers and visualised on an A3130*xl* sequencer (both Applied Biosystems, Darmstadt, Germany). D-loop sequences were aligned using ClustalX (ver.2; Thompson et al. 1997; Larkin et al. 2007) and controlled visually. In order to align the obtained sequences to already known roe deer haplotypes we retrieved the sequences of the 49 haplotypes described by Vernesi et al. (2002) from GenBank. Haplotype cluster assignment was carried out using the neighbour joining option implemented in MEGA (v.4.0. Tamura et al. 2007) based on the clusters proposed by Vernesi et al. (2002).

To genotype the three different source materials of each roe deer we used a panel of 12 microsatellite loci: BM4107 (Bishop et al. 1994), HAUT14 (Thieven et al. 1995), NVHRT16, -21, -24, -30, -48 (Røed and Midthjell 1998), and Roe01, -05, -06, -08, 09 (Fickel and Reinsch 2000). One primer of each pair was 5′-labelled with a fluorescent dye (6-FAM or HEX). We used two reaction mixtures. Mixture I (used for Roe01, Roe05, Roe06, Roe08, BM4107, and Haut14) contained: 0.18 μl FastStart Taq DNA polymerase (5U/μl; Roche), 1.5 μl 10× reaction buffer (containing 20 mM $MgCl_2$), 1 μl BSA (2 μg/μl), 0.75 μl dNTPs (10 mM each), 1.2 μl of each primer (10 pmol/μl), 3 μl DNA (50-120 ng) in a final volume of 15 μl. Mixture II (used for Roe09 and all NVHRT-loci) consisted of 0.07 μl FastStart Taq DNA Polymerase (5 U/μl, Roche), 1 μl 10× reaction buffer (containing 20 mM $MgCl_2$), 1 μl BSA (2 μg/μl),

		Locus and DNA source material											
ID	Haplo-type	BM 4107	Haut 14	NVH RT 16	NVH RT 21	NVH RT 24	NVH RT 30	NVH RT 48	Roe 01	Roe 05	Roe 06	Roe 08	Roe 09
		H F	H F	H F	H F	H F	H F	H F	H F	H F	H F	H F	H F
BW01	HT50	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○
BW02	HT30	○○	○○	○○	○○	○○	○✖	○○	○○	○○	○○	○○	○○
BW03	HT28	○○	○○	○○	○○	○○	○✖	○○	○○	○○	○○	○○	○✖
BW04	HT51	○○	○○	○○	○○	○○	○●	○○	○○	○○	○○	○○	○✖
BW06	HT30	○●	○○	○○	○○	○○	○●	○○	○○	○○	○○	○○	○○
BW07	HT14	○○	○✖	○✖	○✖	○✖	○✖	○✖	○○	○○	○○	○○	○○
BW08	HT30	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○
BW09	HT51	○○	○○	○○	○○	○○	○○	○○	○○	○○	○✖	○○	○○
BW10	HT30	○○	○○	○○	○○	○○	○✖	○○	○○	○○	○○	○○	○○
BW11	HT53*	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○
BW12	HT30	○○	○○	○✖	○○	○✖	○✖	○◘	○○	○○	○○	○○	○○
BW13	HT14	○✖	○✖	○✖	○✖	○✖	○✖	○○	○○	○✖	○✖	○○	○✖
BW14	HT30	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○◘
BW15	HT14	○○	○○	○○	◘○	○○	○○	○○	○○	○○	○○	○○	○○
BW16	HT16	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○
BW17	HT30	○○	○✖	●✖	○○	○○	○○	○○	○○	○○	○○	○○	○○
BW19	HT30	○○	○✖	○○	○○	○○	○○	○○	○○	○○	○○	○✖	○○
BW20	HT30	○○	○○	○○	○○	○●	○○	○○	○○	○✖	○○	○✖	○○
BW21	HT14	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○	○○

Fig. 1. Roe deer genotypes retrieved from hair and faeces compared with control genotypes from tissue across 12 microsatellite loci. Haplotype (HT) numbering follows Vernesi et al. (2002). * HT53 was assigned to HT-cluster A1, while all other HTs belonged to HT-cluster A3 (Vernesi et al., 2002). DNA source material: H – hair, F – faeces. Genotyping errors in non-invasively collected DNA source material were detected by comparison with control genotypes retrieved from tissue samples of the same individuals. ✖ – PCR failed (missing data), ○ – no differences to control (tissue) genotype, ● – differences to control genotype (allelic dropout), ◘ – differences to control genotype (false allele).