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Short communication

Detection dogs allow for systematic non-invasive collection of DNA samples from Eurasian lynx

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ABSTRACT

As Eurasian lynx (*Lynx lynx*) show signs of population recovery in parts of Central Europe, sound monitoring strategies are required to study population expansion, connectivity and genetic diversity. While non-invasive DNA sampling strategies could serve this task, genetic samples of lynx are generally hard to locate. To test the suitability of dog-based sampling we searched scat samples of lynx in the Bavarian Forest National Park, Germany, with two trained detection dog teams. In 44 grid cells of 2 × 2 km, dog teams covered 440 km of predetermined forest road and hiking trail transects during the four week survey. A total of 169 collected samples resulted in 52 genetically confirmed lynx detections, of which 26 were assigned to 11 individuals. Using a single-season site occupancy model we found a detection probability of 0.13/km (SD = 0.02), with 10 km of dog search per grid cell required to get a 70 % probability to detect lynx presence. Our results show that detection dogs are an appropriate tool for systematic genetic lynx monitoring. We argue that detection dog-assisted genetic monitoring may supplement monitoring strategies based on conventional camera trapping, especially when aiming to monitor genetic diversity and population connectivity.

Large carnivores such as wolves (*Canis lupus*), brown bears (*Ursus arctos*) and Eurasian lynx (*Lynx lynx*) are currently recolonising parts of their historic range in Western and Central Europe (Chapron et al., 2014). This population expansion is intensively monitored in these species, due to their high societal and environmental impact. In the case of wolves and bears, traditional monitoring methods are routinely complemented by the genetic identification of species, populations and individuals based on non-invasively collected samples (e.g. Barba et al., 2010; Lucchini et al., 2002). Interestingly, only few studies have successfully applied genetic approaches in monitoring programmes of Eurasian lynx (Bull et al., 2016; Davoli et al., 2013). Lynx are elusive and single individuals cover large home ranges, which poses a challenge to monitoring schemes (Weingarth et al., 2012). Experts demand that monitoring should be intensified and recommend genetic monitoring alongside with camera trapping (Boitani et al., 2015; Chapron et al., 2014). Currently, standardised lynx monitoring is mainly conducted by camera trapping, which is a very efficient method for detecting lynx presence and calculating population densities (Weingarth et al., 2015). However, with this technique discrimination of single individuals requires distinct fur patterns, which are not reliably existent in all subpopulations (Thüler, 2002). Furthermore, information regarding genetic population structure, genetic diversity, inbreeding, ancestry and kinship cannot be acquired with photo-monitoring.

DNA samples from lynx are notoriously difficult to obtain in their natural habitat. Lynx scats are hard to find and cannot be reliably identified in the field (Alda et al., 2008). There have been approaches using hair trapping, but success rates were generally low (Schmidt and Kowalczyk, 2006). Snow tracking is an option to gain scat samples. The effort for this method is high and snow cover is sparse in many areas, such as the Central European low mountain region where the lynx is currently expanding its range. Especially in recently populated areas with low lynx density, however, effective systematic monitoring strategies are required, including the

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genetic assignment of dispersing animals to source populations as well as estimating genetic connectivity between isolated population fragments.

The use of trained dogs in wildlife management and conservation to obtain information about one or more target species has gained increasing attention among researchers and conservation managers during the last years (Dahlgren et al., 2012; Woollett et al., 2014). Scat detection dogs have successfully located genetic samples of closely related species, i.e. bobcat (*Lynx rufus*) (e.g. Clare et al., 2015) and Canada lynx (*Lynx canadensis*) (Mumma et al., 2015). To our knowledge, however, no study has been published so far on scat detection dog use for surveying Eurasian lynx, which occupy considerably larger home ranges than the new world lynx species.

In this study we therefore investigated whether the use of detection dogs is a feasible method for a systematic genetic lynx assessment. We combined the detection dog survey with camera trapping in the Bavarian Forest National Park (BFNP) (see Fig. 1a), which hosts a well-monitored reintroduced lynx population (Weingarth et al., 2015) to generate a sophisticated estimate of detection probability for the dog-based approach.

The BFNP is Germany's first national park founded in 1970. It covers 240 km^2 on the German side and is adjacent to the Czech Šumava National Park. Elevation ranges from 600 m to 1453 m a.s.l. and annual mean temperatures are from 2 °C to 6.5 °C. The forest is dominated by *Picea abies* mixed with *Fagus sylvatica* and *Abies alba* (Cailleret et al., 2014). The current lynx population in the German-Czech border region is based on reintroductions in the 1970s and 1980s on both sides (Wölfl et al., 2001), with a current population estimate of 59–83 individuals (Wölfl et al., 2015).

We set up a 2×2 km grid across the entire BFNP area. We selected grid cells that are 80 % or more within the BFNP borders, which resulted in a total number of 44 adjacent grid cells (Fig. 1b). Two detection dog teams consisting of one handler, dog and an orienteer each conducted scat surveys from 25.03.2017 to 21.04.2017. While both handlers worked with their individual dogs, one additional person per dog team ensured navigation in the field. Both teams surveyed on predetermined transects that were preferably circular (Fig. 1a), for logistic reasons. Transects were mainly along forest roads or paths. We targeted to cover transects only once in one direction and only by one team. Each team covered 22 transects and was required to survey each of the 44 grid cells. Dogs and orienteers were equipped with GPS devices (Garmin Alpha 100 T5 Bundle for team 1; Garmin GPSMAP 64 s and eTrex 10 for team 2) in order to track the distance covered. Dog 1 (female Labrador Retriever) was 11 months old with seven months of previous scat detection training. It mainly searched on a 15 m leash and indicated a find through a bringsel, which is an item attached to the collar that is taken into the muzzle by the dog in reaction to a find. The dog was rewarded with food and a subsequent short game with a toy. Dog 2 (female Border Collie Golden Retriever cross) was four years old with 14 months of training. It searched off-leash and indicated by freezing and staring at the sample. This dog was rewarded through playing with a ball. Both dogs, who have been living with their handlers from puppy age on, were trained based on positive reinforcement by their handlers using samples from numerous captive and free-ranging lynx (Wasser et al., 2004). Though blind testing of dog teams had been included in training sessions, no formal assessment of accuracy was conducted prior to this study. Consequently, dog accuracy could potentially differ between dogs. After a find, sample-related information including environmental data and specific dog behaviour related to the detection were recorded. All samples that dogs indicated on were collected independently of morphological characteristics. Samples were stored in DNA-free 50 ml plastic containers (Sarstedt, Mawson Lakes) with 30 ml of 96 % ethanol and were kept cool until further processing.

We set up one camera trap in every grid cell, for four consecutive weeks. Spatial array of camera traps approximated the standard monitoring design of the BFNP (Weingarth et al., 2012). Duration of camera trapping was set to be broadly congruent to the use of detection dogs. Cuddeback (De Pere) models Capture, C1 and Attack were used in white flash mode. Cameras took one picture per trigger with no pre-set delay. Recovery time was 1.5 s (C1) or 2.0 s (Capture, Attack) during daytime and 10 s during nights. Cameras were attached to trees or wooden poles facing linear structures such as forest roads (Weingarth et al., 2012), tested after setup and checked weekly.

DNA extractions were performed using QIAcube automated DNA extraction devices (Qiagen, Hilden) in a clean laboratory dedicated to the handling of non-invasively collected material. DNA extraction from scat samples was conducted using the QIAamp DNA Stool Kit and a final elution volume of 120 μl. DNA from hair samples additionally found by dogs at scent marking sites was extracted using the QIAamp DNA Investigator Kit (Qiagen, Hilden) as described in Steyer et al. (2016), with two consecutive 40 μl elution steps. Three sequence fragments targeting the mitochondrial control region were used for species identification and haplotyping (see Suppl. 1). A 180 bp stretch (primers L16782 and H16922, Gugolz et al., 2008), a lynx-specific 248 bp fragment (Lynxfwd4 and Lynxrev5) as well as a 234 bp fragment (Hcarn200 and CanidC1) suitable for mammal species identification (Nowak et al., 2014) were sequenced to reveal species identity and control region haplotypes (see Suppl. 1 for reaction conditions). Sequences were assigned to haplotypes described in Gugolz et al. (2008). For confirmed lynx samples individual assignment was performed using a set of 19 microsatellites and two sex markers (Suppl. 1) with three PCR replicates per sample (see Suppl. 1 for details on fragment length analysis). Consensus genotypes were derived using a custom R script (R Core Team, 2016) based on the algorithms used in GIMLET 3.3 (Valière, 2002). Consensus genotypes were assigned to individuals using a customized R script under consideration of gender, haplotype, sampling date and location. A maximum of three mismatching loci was accepted to assign a sample to the same individual due to high error rates in non-invasive samples. Amplification success, allelic dropout and false alleles were calculated using GIMLET 3.3. (Valière, 2002). Only lynx samples with an amplification rate of >0.4 across loci and replicates were considered for further processing.

We used a single-season site occupancy model to estimate the power of the dog-based survey method. The model (Suppl. 2) was implemented in WinBUGs using the package R2WinBugs according to Kéry et al. (2012) with 20.000 iterations, 3 chains, a burn-in of 4.000 and a thinning of 5. Furthermore, we extended the model by a second detection process based on camera traps. Therefore occupancy estimation was based on both camera traps and detection dog survey. The data was subdivided into spatial replicates (1 km transect segments) for the detection dog survey and temporal replicates (one week) for the camera traps.

On 27 days one or both teams conducted detection dog surveys, resulting in 43 team search days during which 440.3 km of transects were covered. Excluding breaks and data recording times, teams searched for 149.2 h. For more details regarding total and daily distances covered by handlers and dogs, total and daily time in the field and actual search time see Table 1.

The two detection dog teams found 165 scat and four hair samples. Fifty scat and two hair samples (30.8 %) showed lynx haplotypes, while 78 (46.2 %) yielded DNA of other species. For 23.1 % of all samples no species could be identified. The dogs were trained for and successfully detected scats of two further species, namely wolf (*Canis lupus*) and European wildcat (*Felis silvestris*). Therefore, false positive indication rate (=42.6 %, mainly fox (*Vulpes vulpes*)