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Dogs can scent-match individual Eurasian beavers from their anal gland secretion

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Dogs *Canis lupus familiaris* are increasingly being used in wildlife conservation studies, due to their extensive ofactory capabilities. Dogs are a useful tool for species detection, species discrimination (or subspecies), and scent-matching of individuals within a species. Scent-matching can reduce or eliminate the need for expensive genotyping of obtained biological samples. We investigated the potential use of dogs to scent-match individual Eurasian beavers *Castor fiber* via anal gland secretion (AGS) samples, in 30 double blind floor platform experiments. We hypothesised that dogs can scent-match individual beavers when presented with AGS from different beavers of both sexes. We showed that dogs were able to scent-match individual beavers with average accuracy of 88.9%, sensitivity of 66.7% and specificity of 93.3%. Our results suggest that scent-matching dogs may be used as a reliable additional method to DNA analysing of biological samples to improve accuracy of individual beaver detection, and a better alternative than live-trapping/capturing in monitoring of specific beavers in e.g. a reintroduction project.

Keywords: anal gland secretion, *Castor fiber*, dogs, non-invasive methods, scent-matching

Due to their highly sensitive olfactory system, dogs *Canis lupus familiaris* have been used in a variety of scent-detection tasks for thousands of years, and their application has significantly increased during the latter years (Rosell 2018, DeMatteo et al. 2019, Bennett et al. 2020). Dogs have been used to locate live or dead animals, their dens, nests or lairs and their signs, tracks and scats. Dogs have also been used in the scat detection of a range of species, e.g. tuatara *Sphenodon punctatus*, Marlborough green gecko *Naultinus manukanus* and forest gecko *Hoplodactylus granulatus*, grouse *Lagopus* spp., North Atlantic right wales *Eubalaena glacialis*, grizzly bear *Ursus arctos* ssp., Eurasian lynx *Lynx lynx*, amur tiger *Panthera tigris* and many others (Rolland et al. 2006, Woollett et al. 2014, Browne et al. 2015, Rosell 2018, Arnesen et al. 2020).

A possible method for monitoring individual mammals could be the use of scent matching dogs (Kerley and Salkina 2007, Wasser et al. 2009). By using rigorous training methods, dogs can achieve very high sensitivity (the proportion of positives that are correctly indicated as such) and specificity (the proportion of negatives that are correctly indicated as such) for human scent-matching (Pinc et al. 2011, Marchal et al. 2016, Hale 2017). This canine-based individual scent-matching has been applied to wildlife studies by using their scats (Kerley and Salkina 2007, Wasser et al. 2009) but no wildlife studies have investigated other sources of scent material in scent-matching work.

The 'remote scent tracing (RST)' method (Fjellanger et al. 2002) entails collecting scent samples at locations where dogs cannot be deployed efficiently for safety, environmental or logistic reasons. These scent samples are then presented to the dogs in a laboratory-like setting for analysis. Authors have brought field samples from for examples mines and corrosion in to the laboratory (Fjellanger et al. 2002, Schoon et al. 2014). The laboratory method has proven to give many advantages, as for example controlled microclimate, optimised scent perception by immediate delivery of rewards and controlled and familiar environment (Gadbois and Reeve 2014).

The Eurasian beaver *Castor fiber* was once spread throughout Europe and Asia but they were reduced to approximately 1200 individuals at the beginning of the 20th century. The main cause for the species' near disappearance was overhunting (Nolet and Rosell 1998). Today, beavers have been reintroduced to many countries in Europe, and the population size is currently estimated to be 1.04 million. The species was reclassified by IUCN to Least Concern in 2008 (Halley et al. 2012). However, due to the fact that beavers

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are keystone species in freshwater ecosystems (Rosell et al. 2005), Eurasian beaver distribution was recovered through widespread translocations and reintroductions, relaxation of persecution and natural spread (Halley et al. 2012).

Both the Eurasian beaver and North American beaver *C. canadensis* are territorial, semi-aquatic, crepuscular and nocturnal mammals (Wilsson 1971, Novak 1987) living in organised colonies (families) usually consisting of adults and their offspring (Nolet and Rosell 1994). The beavers' main communication system is olfactory communication (Campbell-Palmer and Rosell 2010). Due to their strict territoriality, they rely on scent to deter potential intruders (Rosell and Nolet 1997, Schulte 1998, Rosell et al. 1998). The beaver uses two pairs of organs for scent marking (Walro and Svendsen 1982, Rosell and Sundsdal 2001). These are located in two cavities between the pelvis and the base of the tail and consist of two castor sacs and two anal glands. The anal gland is a holocrine secretory gland producing AGS (anal gland secretion). Both castoreum and AGS are secreted onto small piles of mud and debris close to the water's edge, which are easily found in the field (Walro and Svendsen 1982, Rosell and Bergan 2000, Rosell and Sundsdal 2001). Eurasian beaver female AGS is a thick grey paste, whereas male AGS is a yellowish oily fluid (Rosell and Sun 1999). Previous studies have shown that beavers' AGS codes for a variety of information, such as species (Rosell and Sun 1999, Rosell 2001), subspecies (Rosell and Steifetten 2004), sex (Rosell and Sundsdal 2001, Cross et al. 2014), individuality (Sun 1996, Tinnesand and Rosell 2012), kinship (Sun and Müller-Schwarze 1997, 1998), age and social status (Tinnesand et al. 2013).

Until now, one of the most common methods to monitor beavers on an individual level is live-trapping/capturing. Live-trapping provides a large amount of information about individuals (Taberlet et al. 2001), and provides DNA material (hair, tissue, blood) for subsequent genetic analyses (Herr and Schley 2009, Frosch et al. 2011). The most common methods to capture beavers are the use of cage traps (Rosell and Kvinlaug 1998) and trapping with landing nets (Rosell and Hovde 2001). An alternative method to collect beaver DNA, without subjecting them to the stress of capture and handling, is the use of barbed wire hair traps (Herr and Schley 2009). Samples from barbed wire hair traps can be used to determine the number of individuals present in the area. In addition to these methods camera traps are particularly useful for species that are individually identifiable (Di Cerbo and Biancardi 2013) but in general beavers are not (if not ear-tagged) (Campbell-Palmer et al. 2015).

In this study, we investigated the potential to use dogs as an additional tool to scent-match individuals of Eurasian beaver via AGS. We hypothesize that dogs are able to discriminate individual beavers by detecting the individually-unique odours in beaver AGS. We predicted that the dogs would alert to the matching beaver AGS sample and ignore the control samples of other beavers.

Methods

The AGS samples were collected during March–November between 1999 and 2015 from individual Eurasian beavers as a part of a long-term study carried out by the University of South-Eastern Norway. All 75 AGS samples used in this study were collected from live trapped beavers, using landing nets (Rosell and Hovde 2001), in the rivers Straumen (59°29′N, 09°153′E), Gvarv (59°38′6″N, 09°17′9″E) and Saua (59°44′4″N, 09°30′7″E) in Telemark County, south-eastern Norway. To collect the AGS samples, the tail was lifted and the rectum emptied. The cloaca area was then rinsed with distilled water and the papillae of the anal gland were pushed out separately and the AGS squeezed out. Live-trapped beavers were sex-determined by the colour and viscosity of their AGS (Rosell and Sun 1999, Cross et al. 2014). The AGS was placed in glass vials with teflon lids and stored in a freezer at −20°C until used. According to Sun (1996), freezing and thawing of AGS does not significantly affect its composition and smell.

Dogs and scent donors

We trained three privately owned border collies (named Tapas, Chilli and Shib; Table 1). All dogs had previously been used for scent detection work and therefore were familiar with both the table and floor platforms used for scent training and experiments (Fischer-Tenhagen 2011). The dogs had previously been taught to independently investigate and indicate the position of a target sample by lying down and pointing with their nose or paw. All three dogs were trained off-leash. Neither of the female dogs were in heat during training or the experiment. We used two non-professional dog handlers with a scientific background, and with some dog handling experience, e.g. they both participated in the study by Rosell et al. (2019).

We used 75 individual beaver target and control (distraction) samples (41 males, 34 females) of different age groups (average age males = 3.2 ± 3.7 SD; average age females = 2.8 ± 1.1 SD) divided into training (35) and testing (40) samples. For training, we divided the AGS sample of each individual into two 20 ml headspace glass vials with teflon lids (20 ml EPA Vial) each containing 0.1 g of AGS.

Table 1. The dogs (border collies) trained for the experiments (10 trials per dog), age at final experiment, sex (M: male; F: female), handler (both non-professional but with some experience) and results (TP-true positive, FP-false positive, TN-true negative, FN-false negative, sensitivity TP/(TP + FN), specificity TN/(TN + FP) and accuracy (TP + TN)/(TP + FP + FN + TN)). All experiments were carried out on 25 May 2016 in Bø in Telemark.

Dog	Age	Sex	Handler	TP (n)	FP (n)	TN (n)	FN (n)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Precision
Tapas	8.5	M	A	8	2	48	2	80.0	96.0	93.3	0.8
Chilli	8.5	F	B	6	4	46	4	60.0	92.0	86.7	0.6
Shib	11	F	B	6	4	46	4	60.0	92.0	86.7	0.6
All				20	10	140	10	66.7	93.3	88.9	0.7

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We prepared distraction AGS samples from other beavers in the same manner. To avoid contamination during sample preparation, we used sterile gloves and stainless-steel spatulas and sterilised previously used equipment with a gas jet, and cleaned it with 96% ethanol and distilled water. Before training, we removed the samples from the freezer to thaw in room temperature (≈ 17°C) for a period of approximately 20 min.

Laboratory and equipment setup

We carried out all training and experiments in a laboratory at the University of south-eastern Norway, Bø in Telemark, Norway. The laboratory had two separate rooms; one for training (only one dog was present in the training room at the time), and one for the dogs to relax in their crates between training sessions. The experimenter sat in the corner of the training room and faced in the opposite direction of the scent line-up, preventing visual contact between the experimenter, dog and handler (Fig. 1).

The training platform we used was the same as in previous training of the dogs (Fischer-Tenhagen 2011) following recommendations for dog training and experimental design by Johnen et al. (2017). We performed training using a floor platform which is usually used for scent detection tasks (Johnen et al. 2013). The floor platform was placed on the ground and consisted of two sections, each with three sample slots (together six sample slots) that were 30 cm apart.

In each scent line-up, we presented the dogs to a search sample, a matching target sample and 1–3 distraction samples from different beavers. Blank samples were represented by empty glass vials. We placed each vial into a plastic cup. The searched sample was placed on the ground in a separate small platform (smell platform) which consisted of one sample slot, while the target sample of the same individual was placed in the floor platform. The distance between the smell platform and the floor platform was approximately two meters (Fig. 1).

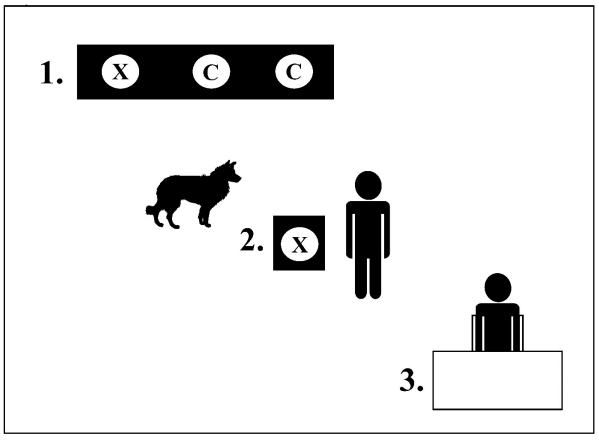


Figure 1. During the training stage one we used one half of the floor platform with three sample slots (X = target AGS sample, C = control/distraction samples) (1); Smell platform with searched AGS sample of the same individual (X) (2); and Experimenter's seat facing the opposite direction of the scent line-up (3).

Training

We carried out 54 training sessions between September 2015 and May 2016. Training took place in the morning, three times a week (Monday, Wednesday, Friday) and lasted approximately 25 min for each dog. Each session consisted of 10 trials for every dog. The intensity of trials and length of breaks were adjusted to the performance and focus of the dogs. This helped us sustain their motivation and their activity.

After each trial the experimenter changed the samples and their positions whilst the handler stood unaware of the target scent location. Samples were assigned a position in the scent line-up randomly by using a die. In between each trial, the platforms were cleaned thoroughly with 7% vinegar spray to remove scents left by previous dogs (Arendash et al. 2001, 2006). Due to vinegar being an aqueous solution of acetic acid it easily bonds with volatile molecules and thus was used to remove the scents of the previous dogs. The study room were further ventilated to aid in diffusion, with doors being left open.

In accordance with previous studies, we based training on positive reinforcement (Deldalle and Gaunet 2014) using a clicker as a confirmation of the correct response (McGowan et al. 2014). We also used a food treat reward alongside vocal encouragement and petting (Feuerbacher and Wynne 2015), immediately after a dog indicated the target. The food treat rewarding took place at the target scent location and was placed on the surface of the platform next to target sample (Kiddy et al. 1978, Fjellanger et al. 2002, Kauhanen et al. 2002).

Initial training (phase one) consisted of two stages. For stage one, we used one half of the floor platform, i.e. one platform with three sample slots were presented to the dogs (Fig. 1). The main goal was to refresh the dogs' memory of the floor platform and to introduce them to AGS samples. The dogs had to identify one target sample (AGS) out of three samples (one target and two blank samples). The dog began by sitting next to the handler who then encouraged the dog to sniff the AGS sample in the smell platform with the command 'smell!' whilst the handler pointed with his hand to the surface of the smell platform. Handler subsequently encouraged the dog to investigate the scent line-up with the command 'search!'. During the first three trials, we placed the target scent at the end of the line-up to encourage the dogs to sniff all the samples in the floor platform. The handler accompanied the dog to the scent line-up and encouraged the dog to investigate all the samples starting at the opposite end of the floor platform from the target scent location. After sniffing the two blank samples, the dog indicated the position of the target scent at the end of the line-up by lying down and pointing with nose or paw. Handler rewarded the dog for every correct indication. After the first three trials the position of the target scent was changed by the experimenter and the process was repeated until each dog independently sniffed the smell platform and found the target sample in the floor platform without the handlers help. Until now, the handler was aware of the location of the target sample.

Phase one (one half of the floor platform) progressed to stage two where we added distraction samples to the floor platform. The distraction scents consisted of: 1 g coffee, 1 g

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tobacco or 1 g herbal tea. During stage two the handler was blind to the position of the samples (single blind) and a correct or wrong response was confirmed by the experimenter vocally. If the dog performed, an incorrect response the trial was marked as incorrect and if the dog gave a correct response the trial was marked as correct. Training progressed to phase two once the dogs accomplished 9 correct indications in 10 consecutive trials (90%) (Wasser et al. 2004).

In phase two (one half of the floor platform) (individual scent-matching), we added an AGS sample from another beaver as a distraction scent. At first, we used an AGS sample of a female individual as a distraction scent and a male as the target scent. Using the opposite sex made the scent-matching easier for the dogs as AGS composition differ between the sexes in beavers (Rosell and Sun 1999). The donor samples (target and distraction) were changed after every trial, as well as the position of the samples. During phase two the handler was unaware of the location of the target sample (single blind). This first stage of individual scent-matching was accomplished after the dogs achieved 9 correct beaver indications in 10 consecutive trials (90%).

Phase two progressed by including the second half of the floor platform. Six samples were now presented to the dogs in the floor platform. The line-up consisted of the target sample and distraction samples from two different opposite sex beavers, a tea sample, a coffee sample and a blank control. Distractions scents from other animals were not included since our dogs have been shown not to react to them (Rosell et al. 2019). After the dogs adjusted to the extended platform, we added a third AGS distraction scent, i.e. a beaver sample of same sex as the target scent. The dogs had to identify one target sample out of four AGS samples (0.1 g), one distraction sample (0.1 g herbal tea or coffee) and one blank sample. The second phase of individual scent-matching was accompliseh after the dogs achieved 9 correct beaver indications in 10 consecutive trials (90%).

Experiment evaluating individual scent-matching accuracy

We carried out the scent-matching experiment on the 25 May 2016. We used the full floor platform (six samples) consisting of four AGS samples (one target, three beaver distractions), one distraction sample (tea or coffee) and one blank sample. AGS samples used in these experiments had not been used during training, and the sex and age group of beavers were chosen randomly. We carried out 30 randomised and independent trials (i.e. 10 per dog). Between trials, we cleaned all equipment with vinegar solution and used a new sterile plastic cup for every sample. The dog handler was blind to the position of the samples and the position of the samples were assigned randomly by an experimenter not involved in the training (Cornu et al. 2011, Johnen et al. 2013). Correct trials were confirmed by the experimenter who was present in an adjacent room (i.e. double blind experiments). We recorded all trials with a tripod mounted videocam set in the corner of the room covering both the smell and floor platforms.

The experimenter recorded the dog's responses as: 1) the dog indicated the target AGS sample – true positive (TP). 2) The dog did not indicate a distraction or control sample – true negative (TN). 3) The dog indicated a distraction or control sample – false positive (FP). 4) The dog did not indicate the target AGS sample – false negative (FN) (Fischer-Tenhagen 2011).

From these responses we calculated four parameters: sensitivity, specificity (Concha et al. 2014, Jezierski et al. 2014), accuracy (Marchal et al. 2016) and precision (Bennett et al. 2020) to determine whether the dogs can scent-match individual AGS. Sensitivity was calculated as:

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN})$$

specificity was calculated as:

$$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP})$$

accuracy was calculated as:

$$\text{Accuracy} = (\text{TP} + \text{TN}) / (\text{TP} + \text{FP} + \text{FN} + \text{TN})$$

and precision was calculated as:

$$\text{Precision} = \text{TP} / (\text{TP} + \text{FP})$$

Results

Training

During phase one (one half of the floor platform), all three dogs achieved the 90% goal after seven training sessions. This first stage of individual scent-matching (phase two) was accomplished in session number 25 by Tapas, session number 39 by Chilli, and session number 31 by Shib. The second phase of individual scent-matching (phase two) was accomplished in session number 51 by Tapas and Shib, and in session number 54 by Chilli. Percentage curves of the correct indication for each dog (Tapas, Chilli and Shib) during training sessions are presented in Fig. 2.

Final experiment

The dogs correctly indicated the samples at an average precison rate of 0.7, accuracy 88.9%, sensitivity of 66.7% and specificity of 93.3% (Table 1). Precision ranged from 0.6 (Chilli and Shib) to 0.8 (Tapas), accuracy ranged from 86.7% (Shib and Chilli) to 93.3% (Tapas), sensitivity ranged from 60.0% (Chilli and Shib) to 80.0% (Tapas) and specificity ranged from 92.0% (Chilli and Shib) to 96.0% (Tapas) (Table 1). There may be a handler effect since handler A had a higher accuracy than handler B. However, it may also be a sex effect since handler A handled the male dog Tapas, and handler B the two females Ship and Chilli (Table 1).

Discussion

The dogs showed a high rate of scent-matching individual beaver AGS, and thus supported our hypothesis. Our results

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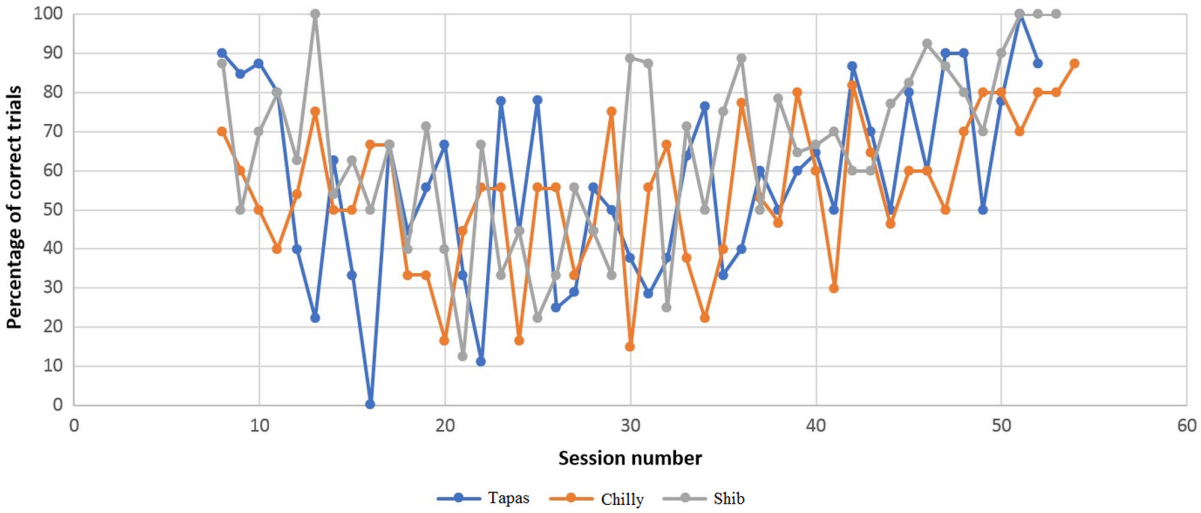


Figure 2. Percentage curves of the correct indication for each dog during training sessions.

suggest that scent-matching dogs may be used as a reliable additional method to DNA analyzing of samples, when there is a need to identify individual beavers. This study also demonstrated that the composition of compounds present in AGS differ between individual Eurasian beavers. This is consistent with chemical analyses on the North American beaver (Sun 1996), and the Eurasian beaver (Tinnesand and Rosell 2012). The most obvious explanation to account for the difference in chemical composition would be the differences in their genetics (Sun and Müller-Schwarze 1997, 1998). Diet is most likely not affecting the AGS since it is a gland (Svendsen 1978). The existence of individual-specific odours have been established in a wide variety of mammals due to their differences in genetics, microorganisms and diet (Müller-Schwarze 2006, Wyatt 2014), for example, spotted hyena *Crocuta crocuta* and European badger *Meles meles* have individual specific scent profiles that vary slightly in composition throughout the year (Buesching et al. 2002, Burgener et al. 2009).

Ideally, dogs should achieve a very high success rate (>95%), however in reality such a high detection accuracy is regarded as exceptional and depends on many factors e.g. handlers, number of dogs, length of training, source of odour, number of samples or trials, distraction scent or method of odour presentation (Jezierski et al. 2014, Gadbois and Reeve 2016, Edwards et al. 2017, Johnen et al. 2017, DeMatteo et al. 2019). It also matters if the tests have been carried out in natural or controlled environments. Results from scent detection dog studies have therefore showed different accuracy. For example, Cablk and Heaton's (2006) used dogs in the wild to survey for the desert tortoise *Gopherus agassizii* and had an overall accuracy of 91%. Smith et al. (2003), in their search for kit foxes *Vulpes macrotis mutica* scats in scent boxes indoors, found that dogs chose the correct scat in every trial (100% success) when the target species was present but were less accurate (67%) at ignoring a distraction scent of red foxes when the target scent was not present. A control study carried out by Cooper et al. (2014) tested 11 dog detection teams on their accuracy to detect bed bugs *Hemiptera cimicidae* in naturally infested apartments. In three separate experiments, the mean (min, max) detection rate was 44% (10–100%) and mean false-positive rate was 15% (0–57%), despite of the fact the teams claimed they can detect bed bugs in a controlled environment with more than 95% accuracy. The low accuracy of trained dogs for bed bug detection suggests that the capability of dogs to determine presence or absence of bed bugs in natural conditions may be more limited than under controlled conditions (Cooper et al. 2014). Although all our tests were carried out in a laboratory environment, there is no reason that dogs cannot also be trained for use in the field (Rosell 2018). However, their success rate in the field needs to be investigated.

Until now, scent-matching dogs have been successfully trained to discriminate individuals from scats of amur tiger *Panthera tigris altaica* (87% correct indication) (Kerley and Salkina 2007) and maned wolf *Chrysocyon brachyurus* (89% correct indication) (Wasser et al. 2009). Unfortunately, none of these studies (including ours) requested the dogs to match scents with no correct choice present in the platform. If negative control trials had been carried out the error rate of dogs would probably have increased (Schoon 1998). However, the dogs we used in our study proved themselves able to do negative control trials in another study (Rosell et al. 2019). Also, to gain further knowledge of the ability of dogs to scent-match individual beavers' AGS, the next step should be to use scent samples from different individuals obtained from different seasons and years.

Training dogs is time consuming and expensive but once the dogs are trained, the results are immediate. Using trained dogs can be cost-effective, e.g. if carried out in co-operation with existing public agencies who already have dogs trained for scent detection operations (Orkin et al. 2016). In a reintroduced area, re-trapping/capturing of individual beavers to find out if they are still present in the area or to follow them for a certain length of time to measure the success of the project, often require intensive field work by use of live-traps (Rosell and Kvinlaug 1998) or landing nets (Rosell and Hovde 2001). In many areas where beavers are difficult to observe, these methods are inappropriate when immediate management action is needed. Goodman et al. (2012) stated that the 'hands-on monitoring' associated with the study trial period of reintroduction programs, should be balanced

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to avoid unnecessary stress on the animals. An alternative monitoring method could be to use scent-matching dogs. Dogs do not necessarily need to be brought into the field but scent mounds samples from a beaver family/territory could successfully be brought to the laboratory, and compared with AGS samples of specific individuals collected prior the release. For example, Fjellanger et al. (2002) vaccumed an area of land suspected to contain mines and collected the scent onto filters, while Schoon et al. (2014) sucked air through drain plugs in the insulation material surrounding the pipes onto filters. The next step should therefore be to conduct training in the field in uncontrolled environments with varying weather conditions. We conclude, that dogs could be used as an additional method to DNA analyses to improve accuracy of individual beaver detection, and as a better alternative than live-trapping/capturing in monitoring of specific beavers in a reintroduction project.

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