SCAT-DETECTION DOGS SURVEY LOW DENSITY MOOSE IN NEW YORK

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ABSTRACT: The difficulty of collecting occurrence and population dynamics data in mammalian populations of low density poses challenges for making informed management decisions. We assessed the use of scat-detection dogs to search for fecal pellets in a low density moose (*Alces alces*) population in the Adirondack Park in New York State, and the success rate of DNA extraction from moose fecal pellets collected during the surveys. In May 2008, two scat-detection dog teams surveyed 20, 4-km transects and located 138 moose scats. In 2011 we successfully amplified DNA from 39 scats (28%) and were able to uniquely identify 25 individuals. Improved storage protocols and earlier lab analysis would increase the amplification success rate. Scat-detection dogs proved to be a reasonable, non-invasive method to collect useful data from the low density moose population in the Adirondack Park.

Moose (*Alces alces*) were nearly extirpated from the northeastern United States in the late 1800s, but have recently undergone natural recolonization in the region (Alexander 1993, Bontaites and Gustafson 1993, Wattles and DeStefano 2011) and the Adirondack Park in New York (Hicks 1993, Reeves and McCabe 1997, Jenkins and Keal 2004). Moose have no natural predators in New York other than possibly black bears (*Ursus americanus*) that prey upon neonatal calves, but concerns about over-browsing of regenerating forests, trampling of vacuum tubing in sugar maple (*Acer saccharum*) stands, and the potential for moose to pose roadway hazards have prompted calls for a hunting season. The recent population decline in Minnesota suggests that moose at the southern extent of their range may face thermoregulatory stress that could possibly translate to poor body condition, malnutrition, and energy loss making them more susceptible to parasites (Lenarz et al. 2010). Although state wildlife biologists recognize the need to understand their population dynamics and structure, moose in northern New York occur at low density in small, widely-scattered groups that challenge the collection of meaningful population data.

Moose biologists from the region met in 2003 to discuss potential research and management methods to study the low density population in the Adirondack Park (Kretser et al. 2014). GPS radio-collaring of 10 females, aerial surveys, deer hunter surveys, and other non-invasive approaches were presented as viable options for studying this low density population. At the time, cost of GPS

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radio-collars and the logistical difficulty in capturing moose were considered prohibitively expensive, especially in this heavily forested region dominated by dense coniferous and mixed forest with minimal road access. Deer hunter surveys began in 2005 with low participation rates, and flyovers occurred when helicopter availability and weather conditions aligned, albeit, not frequently enough to collect robust data. The Wild Center, a local natural history museum, offered pilot funding to test other non-invasive methods, specifically, using scat-detection dogs to collect population data. The initial objective of this study was to assess the use of scat-detection dogs to locate moose scat efficiently as a potential technique to estimate moose abundance in the dense forests of the Adirondack Park.

Measuring occurrence and abundance of a wide-ranging mammal at low density poses challenges for biologists desiring to make informed management decisions (MacKay et al. 2008). At low abundance, the effort required to observe or capture individuals may exceed the resources available to obtain adequate and useful data. Methods such as camera trapping and track stations can supply presence/absence information, and in some cases, information about population structure (e.g., identifying males, females, and juveniles in photographs); however, these methods do not produce DNA samples.

Non-invasive techniques such as hair snares and scat sampling are often good alternatives for obtaining DNA samples. Hair snares work well in situations where bait and lure are used to attract an animal to the site (e.g., Woods et al. 1999), or when surveying areas such as feeding sites or habitat features where species congregate or visit regularly (Kendall and McKelvey 2008). Scat collection does not require luring a species to a specific site, rather, an efficient means of locating scat in a natural setting. Recent studies have used fecal DNA to identify

individuals, evaluate kinship, and describe distributions and sex ratios in wild populations (Taberlet et al. 1997, Lucchini et al. 2002, Eggert et al. 2003, Bellemain et al. 2005). Because human detection of scats is challenging in a low density population, scat-detection dogs are often used to increase efficiency (Smith et al. 2003, Long et al. 2008). Combining DNA analysis with the use of scat detection dogs eliminates the need to capture, handle, or observe individual animals and minimizes the field time required to collect samples (Kohn and Wayne 1997, Kohn et al.1999).

Obtaining DNA from wild animals provides for a variety of uses and approaches to extract relevant population data. Noninvasive genetic samples can be used in a population genetic framework to understand effective population size, gene flow, genetic diversity, and kinship across multiple populations (Schwartz and Monfort 2008). Mitochondrial DNA (mtDNA) can be used to identify individual species (Foran et al. 1997), nuclear DNA (often using microsatellites) can identify individuals, and sex identification is possible by focusing on specific genes that determine gender (Schwartz and Monfort 2008). These data can be obtained from DNA extracted from blood, tissue, hair, or scat. Sampling of high quality template DNA samples (i.e., tissue and blood) is often invasive, requiring physically handling animals which may entail high cost, physiological stress, and/or injury.

To date, most research involving scat-detection dogs has focused on carnivores (Long et al. 2008). We sought to assess the feasibility of using these dogs to search for moose fecal pellets in the Adirondack Park and to determine whether DNA extraction from moose fecal pellets was feasible. Several factors influence whether DNA can be extracted from a scat sample including diet, environmental conditions at collection, storage methods, and the specific extraction

method. This method has been used successfully to empirically address a variety of questions about carnivores (Smith et al. 2005, Beckmann 2006), and other organisms ranging from right whales (*Eubalaena glacialis*; Rolland et al. 2006) to invasive plants (Goodwin et al. 2010). Ungulate scat has been successfully amplified in a different ecosystem where scat remained frozen throughout the study period (Wasser et al. 2011). Our two primary objectives were to 1) evaluate if scat-detection dogs could efficiently locate moose scat in a low density population in the Adirondack Park, and 2) to determine the efficacy of extracting DNA from moose fecal pellets collected in this ecosystem.

STUDY AREA

The Adirondack Park (Park) in northern New York is a 24,000-km² mountainous area with more than 3,000 lakes and ponds and 45,000 km of waterways (Fig. 1). Elevation ranges from 305-1671 m and the dominant forest types are northern hardwood, conifer, and boreal upland forests. Northern hardwoods include American beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniensis*), and sugar maple, with red spruce (*Picea rubens*) - balsam fir (*Abies balsamea*) forests at higher elevations and rare alpine vegetation above 1500 m. More than 280 bird, mammal, amphibian, and reptile species inhabit the landscape, alongside 130,000 full-time human residents in 103 rural communities. Nearly half of the land within the Park boundary is privately owned and managed; the public land is permanently protected from development by the New York State (NYS) Constitution. The local economy is based on year-round tourism, commercial forest industry (private land), and governmental services (Jenkins and Keal 2004). The Adirondack Park Agency oversees and regulates activities on the privately-owned portions of the Park, and management of the

wildlife resources on both public and private land rests with the New York State Department of Environmental Conservation (DEC), including hunting and responding to human-wildlife conflicts.

METHODS
Scat Detection

The Wildlife Conservation Society (WCS) conducted a pilot test of scat-detection dogs in the northern Adirondacks in partnership with Working Dogs for Conservation, Inc. (WDC, Three Forks, Montana, USA). Initially, WCS staff worked with local individuals to locate scat samples from multiple moose throughout the Park. Scats were collected on public and private lands including private parcels undergoing active or recent logging that provided contrast to the protected and unlogged state lands. We sent scat samples to the WDC for training dogs on scents associated with scats that reflected the diet of moose in the Park; dogs were trained for 6 weeks followed by a 2-day *in situ* training.

Maps and aerial photographs were used to establish 20 line transects of ~4 km at each site prior to deployment of the dog team. In the challenging terrain, a 4-km transect was considered a reasonable distance for the team to traverse during a one-day session. Each team included one dog handler, one orienteer, and one dog. Each dog wore a GPS unit attached to a work vest to track their movements; likewise, each orienteer carried a GPS to track their movements. Handlers kept the dogs under voice command within 100 m of the transect, and we measured both human and dog tracks by summing the distance between track points recorded every 15 sec. Each transect was labeled with a unique identifier code and described by date, start time, end time, duration, dog and handler, orienteer, temperature, weather at the start of the transect, human track (km), and dog track (km). We recorded the number of
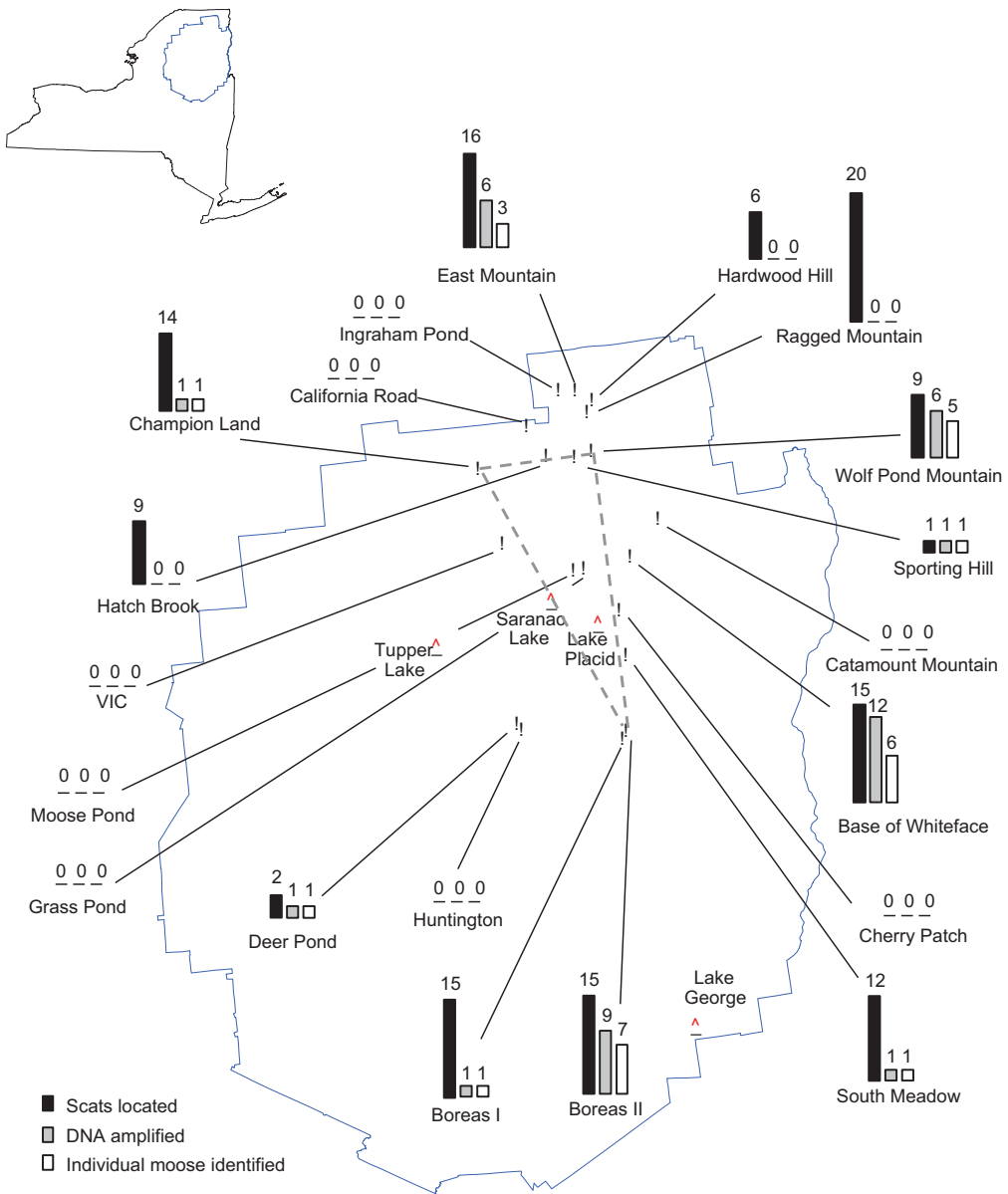
Fig. 1. Map of transects surveyed by working dog crews and the number of scats detected, amplified, and identified as individual moose at each transect. Dotted line denotes straight line path connecting three transects (Wolf Pond, Champion, and Boreas II) where the same individual moose was identified. Inset: Location of Adirondack Park within New York State.

moose scats, bear scats, and unknown scats; we included bear in the survey effort because both dogs were trained previously on black bear scat. Orienteers used latex gloves and plastic Zip-lock bags to collect and store scats in an attempt to maintain a sterile environment and reduce the potential for cross-contamination of samples.

Each scat was assigned a unique identification number and described by species, dog

or human found, date, time, zone, easting, northing, elevation in feet, canopy (i.e., open (>50%) or closed), forest type (i.e., hardwood, softwood, or mixed), and water characteristic of the site (i.e., wetland, within 100 m of a wetland, or upland). We assigned each scat to 1 of 3 condition categories: 1) excellent – pellets well-formed, moist or wet, and dark color (described as "fresh" in Smith et al. 2003, Mondol et al. 2009), 2) good – pellets becoming unformed and starting to have discoloration, or 3) poor-pellets not formed, dry, light in color, sometimes moldy. Scats were stored in Ziploc bags in the field, subsequently transferred to 5 mL plastic vials and covered with ethanol, and stored in a cool dark basement; duplicates were frozen in Ziploc bags. Extremely moist samples were kept in open, brown paper bags (9×16 cm) to air dry (Franzen et al. 1998, Piggott and Taylor 2003) for 24–48 h prior to storage (Maudet et al. 2004). Scats in ethanol were submitted to the United States Forest Service Rocky Mountain Research Station Wildlife Genetics Laboratory (RMRS) in March 2010.

DNA Extraction

Using moose reference samples from the northeast, we optimized a panel of 9 variable microsatellites in an attempt to uniquely identify individuals from the Park (Wilson et al. 2003, Schmidt et al. 2008, Wilson pers. commun.). Reference blood and tissue samples were obtained from collections and sampling of harvested and vehicular-killed moose from 4 northeastern states (New York, New Hampshire, Maine, and Vermont) and 4 Canadian provinces (New Brunswick, Ontario, Nova Scotia, and Quebec). The Nova Scotia samples included moose from the mainland and Cape Breton Island. We performed an initial DNA extraction on 140 fecal pellet samples (stored in ethanol) using a standard protocol developed for ungulate fecal pellets (Maudet et al. 2004, Schwartz et al. 2007). We then

performed a DNA extraction on these samples with a pellet swab to test the efficacy of this approach; after two failed attempts, we repeated this process on frozen duplicate samples (n = 42).

DNA from reference samples was amplified at the following 8 microsatellite loci: NVHRT21, BM1225, BM4516, FCB193, MAP2C, RT5, RT9, and RT30 (Wilson et al. 2003, Schmidt et al. 2008, Wilson pers. commun.). The reaction volume (10 µl) contained 1.0 µL DNA, 1× reaction buffer (Applied Biosystems), 2.0 mM $MgCl_2$, 200 µM of each dNTP, 1 µM reverse primer, 1 µM dye-labeled forward primer, 1.5 mg/mL BSA, and 1U Taq polymerase (Applied Biosystems). The PCR profile was (94 °C/5 min, 94 °C/1 min, 55 °C/1 min, 72 °C/30 s) × 45 cycles. The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). All non-invasive samples were initially amplified twice using the multi-tube approach (Eggert et al. 2003, Schwartz et al. 2004), and allele scores were entered only when consistent for both amplifications. Microsatellite data were checked for genotyping errors (false alleles, allelic dropout and scoring errors) using the program Dropout (McKelvey and Schwartz 2005, Schwartz et al. 2006). Microsatellite data was also error-checked with the program Micro-Checker (Van Oosterhout et al. 2004) to identify loci with possible genotyping errors leading to homozygote excess. We calculated the probability of identity (PID) and probability of identify given siblings (Psib) from these samples.

We used Chi-square with a Cochran's test of linear trend to assess the relationship of scat condition, forest type, and wetland proximity to successful DNA extraction. We then evaluated all possible general linear model (GLM) combinations of these factors in SYSTAT with Akaike Information

Criterion (AIC) to assess their relative importance on our ability to successfully extract DNA.

RESULTS

The 20 transects were sampled on 10 field days in May 2008. Dogs located 191 scats and 4 additional scats were located by the orienteers; 134 (69%) were moose, 56 (29%) bear, and 5 (2%) were unknown. The proportions of moose scat relative to condition were 18% excellent, 45% good, and 36% poor; 63 and 37% of scats were collected on private and state lands, respectively. Dogs traveled 134 km and orienteers 114 km during the surveys.

We genotyped 270 tissues and hair samples from 10 locations at 8 variable microsatellite loci (Table 1). We obtained quality multi-locus genotypes from 28 pellet samples using the initial DNA extraction from fecal pellets. The swabbing method yielded quality DNA from 9 additional pellet samples, and the duplicate samples yielded 2 additional samples. DNA was successfully amplified from 28% (39 of 137) of collected scats using the 8 loci. Errors were identified in 3 samples

at loci RT9 and NVHRT21. DNA from these samples was reanalyzed following the approach of Schwartz et al. (2006) until no errors occurred.

We identified 25 unique individuals. There was a 1 in 9,737 chance of identifying 2 individuals as identical ($P_{ID} = 1.03 \times 10^{-4}$), and a 1 in 64 chance of identifying 2 siblings as identical (Psib = 1.57×10^{-2}). In 8 cases we identified the same moose from multiple scat, and one moose was identified on 3 different transects located >40 miles distant (Fig. 1).

Successful amplification was achieved in 52% of excellent, 31% good, and 12% of poor scats; of the total amplified sample (n = 38), 34% were excellent, 50% good, and 16% were poor scats (Table 2). Scat condition ($\chi^2 = 7.928$, $P < 0.001$) and forest type ($\chi^2 = 7.928$, $P < 0.05$) affected our ability to successfully extract DNA; excellent scats and scats located in hardwoods had the highest success rates. Location was not related to successful extraction (Table 2). The GLM

Table 1. Origin and number of tissue samples received and analyzed to create markers for use in the DNA extraction of moose fecal scats, Adirondack Park, New York.

Country	Location	Received	Used
US	New York	16	16
US	New Hampshire	30	30
US	Maine	41	41
US	Vermont	31	31
CA	Nova Scotia – Mainland	31	29
CA	Nova Scotia – Cape Breton Island	9	9
CA	New Brunswick	46	46
CA	Ontario	29	26
CA	Quebec – RFPL	13	13
CA	Quebec – PLC	29	29
	Total	275	270

Table 2. The rate (%) of successful extraction of DNA (yes = 38, no = 99) from moose scat relative to scat condition, forest type, and microhabitat location in the Adirondack Park, New York. Sample sizes in parentheses.

Variable	Yes	No
Scat condition*		
Excellent (25)	52%	48%
Good (62)	31%	69%
Poor (50)	12%	88%
Forest type[†]		
Hardwood (74)	36%	50%
Softwood (45)	22%	78%
Mixed (18)	6%	94%
Location[‡]		
Immersed in Water (2)	50%	50%
Near water (24)	21%	79%
Upland (111)	29%	71%

*$\chi^2 = 13.782$, $P < 0.001$.
[†]$\chi^2 = 7.928$, $P = 0.019$.
[‡]$\chi^2 = 1.131$, $P = 0.568$.

underscored the relative importance of scat condition and forest types. The top four GLMs included scat condition, with the top model indicating that 69.7% of model weight was associated with scat condition and forest type (Table 3).

DISCUSSION

We demonstrated that scat-detection dogs were effective at locating moose scat in a low density moose population in the dense forests of the Adirondack Park. Scat-detection dogs are more frequently used in carnivore research because of their obvious advantage in sampling wide-ranging and low abundance populations (MacKay et al. 2008); their use in ungulate research is less common. Wasser et al. (2011) used these dogs to locate a variety of species including moose and woodland caribou (*Rangifer tarandus caribou*) in areas proximal to the Alberta tar sands, and successfully extracted DNA from scats of both. Similarly, dogs located scats of a variety of deer species (*Mazama* spp.) in Brazil and outperformed human searchers; humans located zero scats whereas dogs located 0.21 scats/km (de Olivera et al. 2012). The success rate of our dogs was ~1.4 samples/km, confirming our supposition that dogs could efficiently 'sample' a low density moose population which cannot be easily observed/sampled.

Although we successfully amplified DNA from moose fecal pellets, our success rate was relatively low (<30%) but was explained by pellet condition and location. Age and environmental factors (e.g., precipitation, temperature) affect the quality of collected scats (Brinkman et al. 2010), and these factors also affect detection rate (Reed et al. 2011). These factors undoubtedly affected the quality of our samples as we collected scat in the spring, relatively soon after snowmelt. Environmental conditions are different within the 3 forest types, with mixed and softwood stands moister at the forest floor which would presumably degrade scat faster; in fact, extraction rates were higher in scats collected in hardwood forest (Table 2). Collection of fresh scats across seasons and repeat sampling would improve our sampling protocol. For example, in an area of winter concentration of moose, repeat sampling would minimize exposure of fecal pellets to the elements (see Brinkman et al. 2010). Future work may also take advantage of *in situ* photographs of scats to compare condition across sites, and relate condition to amplification success more objectively.

Storage methods and storage time had strong influence on our success rate of DNA amplification. We stored scats in ethanol based on the best information available at the time, and although a common storage method, it was not ideal in our study. Researchers examining relative success rates associated with various storage media hesitate to provide overall

Table 3. Model selection results using AIC for General Linear Models to predict successful DNA extraction from moose scat collected in the Adirondack Park, New York.

Model Rank	Variables	AIC	ΔAIC	AIC weights
1	Scat Condition + Forest Type	157.53	0.00	0.697
2	Scat Condition + Forest Type + Water	159.98	2.45	0.204
3	Scat Condition	162.07	4.54	0.072
4	Scat Condition + Water	164.51	6.98	0.021
5	Forest Type	168.43	10.90	0.003
6	Forest Type +Water	168.65	11.12	0.003
7	Water	175.46	17.93	0.000

recommendations because of inconsistency among studies (Schwartz and Monfort 2008); however, ethanol was considered the worst storage medium among 3 alternatives (Soto-Calderón et al. 2009). The relatively long storage time of our samples (2 years) was probably the major reason for our low success rate with amplification; however, we still recovered DNA from ~25% of the highly variable sample and >50% of excellent pellets. Schwartz and Monfort (2008) suggest processing samples immediately because DNA is more stable in laboratory buffers than fecal material, but the logistical issues of fieldwork would often preclude this approach. However, DNA swabbed from moose fecal pellets and processed within a few weeks of collection yielded high amplification rates (90%; K. Pilgrim, RMRS, unpublished data). Field swabbing of fresh samples also results in higher amplification rates compared to swabbing frozen samples (Rutledge et al. 2009).

Scat-detection dogs offer many advantages but with certain considerations. Cost is a concern for any field-based project and substantial costs are associated with dog and handler selection and training, as well as field time for executing transects (MacKay et al. 2008). In this study we spent $25,000 to hire WDC to find 191 scats, at a per-unit cost of ~$130 per sample. Numerous scats are required to address population studies and depending upon animal density, may require substantial field time and cost. Researchers must understand and work within the physical limitations of the dog and recognize that detection rates often vary among dog/handler teams. The handler must ensure that the dog focuses on the desired scat and is not inadvertently trained to non-target scat; this is particularly salient for handlers when target and non-target species have morphologically similar fecal pellets. Lastly, this method may result in real or perceived potential conflict with wildlife (MacKay et al.

2008), and the presence of dogs in a given environment may result in unforeseen conflicts with local wildlife.

Despite certain limitations, there are numerous and obvious advantages in using scat-detection dogs. In comparison to human searchers, dogs are highly efficient and effective at locating scats (de Olivera et al. 2012), and in this study, covered ~20% more ground than humans and collected all but 4 scats. Dogs create minimal sampling bias (MacKay et al. 2008), allowing for quick confirmation of occupancy of the study area by target species. Collection of scats ultimately provides for discrimination between species and individuals, and has proven applicable to a wide variety of species and habitat types. Collection of scat not only allows for subsequent assessment of population structure, it also provides opportunities to explore additional factors such as stress levels and diet. The charismatic and broad public appeal of using dogs should not be discounted as an opportunity for public outreach and engagement (MacKay et al. 2008, Woollett et al. 2014). One of the lasting impacts of using scat-detection dogs was the creation of two high definition videos describing the project and highlighting the dogs in the field. According to The Wild Center staff, these two films continue to capture audiences largely due to the appeal of the dogs performing in the field.

Our pilot research in the Adirondack Park of New York State is one of a limited number of studies in which scat-detection dogs have been used in ungulate research, and these dogs provided a viable method for sampling a low density moose population. We also found that forest type, the condition of fecal pellets, storage method, and storage time influenced the efficacy of DNA amplification. The impact of these factors can be controlled through improved study design that addresses temporal sampling, field swabbing, shorter storage time, and performing

extractions soon after sample collection. In particular, we recommend swabbing the scats using synthetic swabs (e.g., Dacron Swabs), at least two swabs per sample, and storing them dry in envelopes or vials. Fecal pellets can be stored in vials of 95% ethanol at room temperature, frozen in secure plastic bags, or air dried and kept at room temperature. Ideally, the swabs and scats would be submitted for DNA extraction and analyses within a few days of collection. This approach works for a variety of carnivores and would be an improved protocol for moose research (Reed et al. 2004, McKelvey et al. 2006, Rutledge et al. 2009, Anwar et al. 2011).

Our data and that collected in subsequent and future surveys provide an important foundation to understand habitat use and population dynamics of moose in the Park, and conduct genetic research to determine relationships among Park and regional moose. Given the increased interest and funding available for moose research in New York State, we encourage continued use of scat-detection dogs, in concert with other techniques, to monitor and study the low density moose population in the Adirondack Park.

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