Notes

Timing Moose Pellet Collections to Increase Genotyping Success of Fecal DNA

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Abstract

Fecal pellets can serve as a noninvasive source of DNA for identifying the distribution of individual animals when conducting population estimates. The quality of fecal DNA, however, can be degraded by wet or warm environmental conditions. We tested the effect of time of pellet collection (mid-March–mid-June) and temperature on the success of genotyping microsatellite loci in moose *Alces alces* by using DNA extracted from moose fecal pellets collected from north central British Columbia, Canada. Using the number of microsatellite loci genotyped as a measure of genotyping success clearly indicated that fecal pellets collected in March and April contained DNA that yielded high-confidence genotypes, whereas those collected in May and June did not. Pellets collected in March and April were more likely to be collected in cooler (often subzero) temperatures than those collected in May and June. Pellets collected later in the year were also more likely to be exposed to rain and increased solar radiation, all of which are likely to contribute to degradation of fecal DNA. Our findings suggest that pellets collected in late winter in the Northern Hemisphere have sufficient DNA to permit genotyping of moose.

Keywords: *Alces*; DNA; fecal pellet; genetics; loci; molecular analysis

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Introduction

The collection of fecal DNA is an increasingly popular noninvasive technique for identifying individual animals; establishing the presence of rare or elusive species; determining sex (Fernando et al. 2003; Waits and Paetkau 2005); and examining population structure, mating systems, genetic diversity, and dispersal patterns (Ball et al. 2006; Brinkman et al. 2011). This technique allows for the sampling of a large number of animals quickly and at a lower cost when high-quality fecal samples are available (Wehausen et al. 2004; Waits and Paetkau 2005). For species that are rare, elusive, or difficult to capture, fecal sampling may be the only technique for collecting DNA (Zhang et al. 2006).

Fecal DNA is extracted and isolated from sloughed cells of the intestinal mucosa, which coat the outer surface of the feces. Some investigators consider fecal DNA to be of inferior quality compared to blood and other soft tissues because it can be subject to various

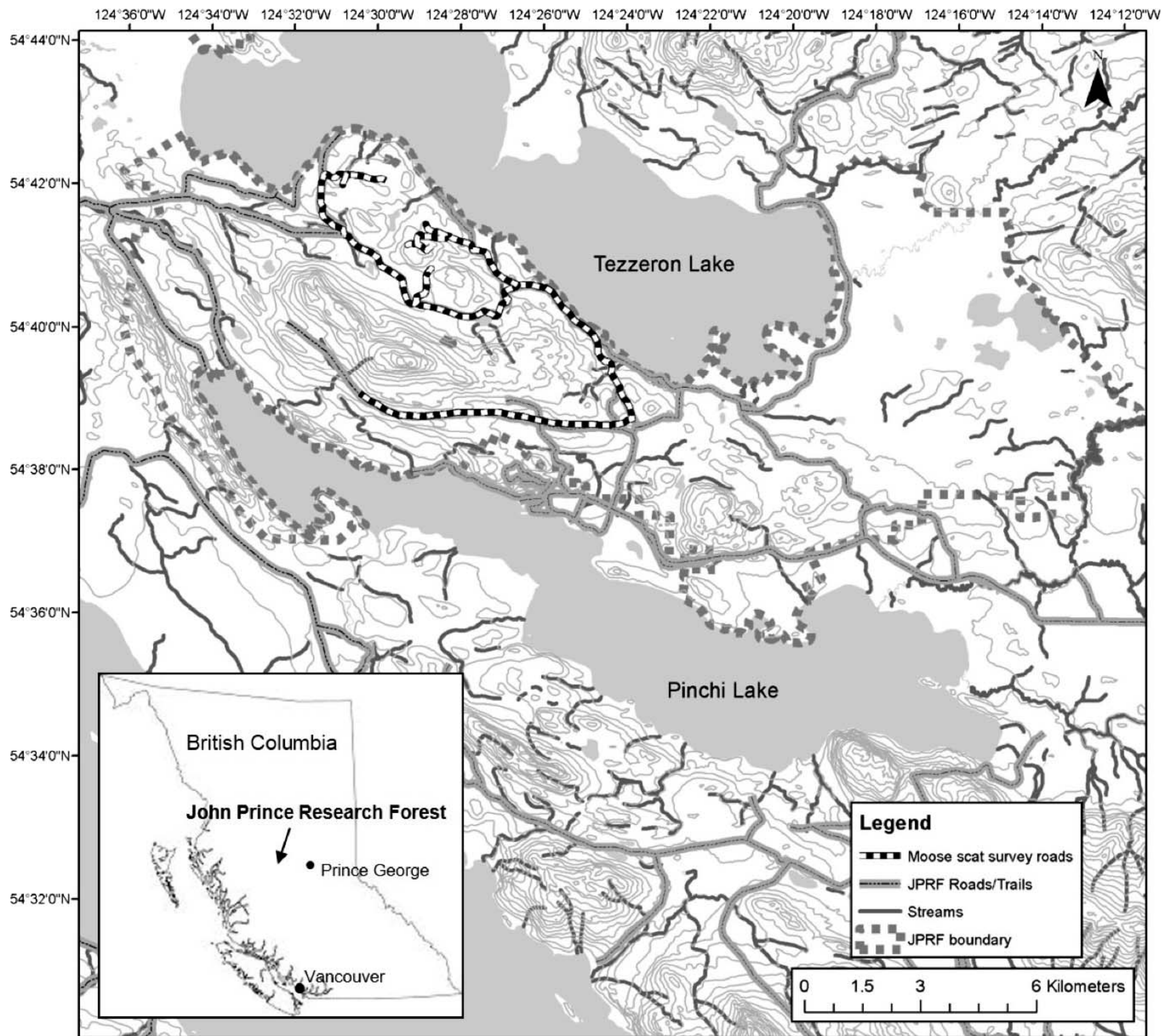

Figure 1. Location of the John Prince Research Forest (boundaries are the gray and white squares) study area in north central British Columbia, Canada, where moose *Alces alces* fecal pellets were collected from March to June 2013 for genotyping. Moose pellet survey lines are delineated with black and white squares.

forms of degradation (Piggot 2004; Ball et al. 2006; Brinkman et al. 2011), whereas others caution against contamination and laboratory error (Fernando et al. 2003). Degradation is known to increase with time since deposition, rainfall, and myriad environmental conditions (Brinkman et al. 2009, 2010). In some cases, those processes are not well understood (Mowry et al. 2011). For these reasons, Maudet et al. (2004) and Wehausen et al. (2004) suggest that more research be conducted to clarify the effect of season and environmental moisture on the genotyping success of fecal DNA collected from ungulates.

Using a suite of eight microsatellite loci and a sex determination marker (ZFX/ZFY; Shaw et al. 2003), our objective was to determine at what time during the late winter and early spring moose pellets should be collected for optimal genotyping success. To this end, we collected fecal pellets of moose during the middle of March (6–12), April (17–22), May (13), and June (19) 2013 to test our null hypothesis that genotyping success would be unaffected by collection date.

Study Site

Moose fecal pellets were collected in the John Prince Research Forest (54°40′10.1′′N, 124°24′52.1′′W; Figure 1). The John Prince Research Forest is a 16,500-ha portion of forested land 45 km northwest of Fort St. James, British Columbia, Canada, and it is co-managed by the University of Northern British Columbia and Tl'azt'en Nation. The area around the research forest is characterized by rolling terrain with low mountains (700–1,267 m

above sea level) and is dominated by vegetation characteristics of the Sub-Boreal Spruce biogeoclimatic zone (Meidinger and Pojar 1991). The area has experienced a wide variety of logging activities over the past 50 y and contains a mosaic of old and young forest with interspersed deciduous stands with a rich understory of deciduous shrubs and regenerating conifers (see Crowley et al. 2012). We obtained weather records for the area from the National Climate Data and Information Archive (Environment Canada 2015).

Methods

Fifteen to 20 moose fecal pellets were collected opportunistically from fecal pellet groups along the road and trail network of the study area (Figure 1). Repeated collections along these routes were possible as snow melted and pellet groups, which had been deposited throughout the winter, became exposed. Moose fecal pellets were easily distinguished from the pellets of elk *Cervus elaphus* and mule deer *Odocoileus hemionus*, both of which are also found in the research forest, by experienced collectors via close examination of the morphological features of the pellets (Hodder et al. 2013). Ten samples of 15-20 pellets each were collected during the middle of each month from mid-March to mid-June 2013 and placed in Ziploc® bags; samples were immediately stored in a −20°C freezer.

Sample bags were removed from the freezer and individual pellets were separated. As recommended by Wehausen et al. (2004), duplicate samples of intestinal mucosal cells were obtained from each sample by gently rubbing the exterior of pellets with porous wooden toothpicks, ensuring that no fecal matter was collected. The toothpicks containing the epithelial cells were then placed in individual paper envelopes left open to dry for 24 h. Toothpicks containing DNA were sealed in envelopes and sent to Wildlife Genetics International (Nelson, British Columbia, Canada) for analysis. There, DNA was extracted by clipping an ~3-mm piece of each toothpick and processing the clippings using the QIAGEN (Toronto, Ontario) DNeasy Blood and Tissue kit, as per QIAGEN's tissue protocol. Using fragment size assays to determine genotypes, the lab personnel amplified and analyzed seven microsatellite loci (BL42, BM1225, BM4513, BM848, OarFCB193, Rt24, Rt30; Buchannan and Crawford 1993; Bishop et al. 1994; Wilson et al. 1997) and a sex (gender) determination marker (ZFX/ZFY; Shaw et al. 2003).

The resulting fragments from all eight amplified loci were analyzed using an ABI PRISM® 310 Genetic Analyzer, and genotypes (i.e., alleles based on fragment size) were scored using Genotyper software (ABI; Foster City, CA. 94404). A single technician scored all data, denoting an allele call as high confidence when it met defined signal intensity thresholds measured using a fluorescent detection method (>100 relative fluorescent units for heterozygous peaks and >600 relative fluorescent units for homozygous peaks) and also met subjective visual cues for strong data (as per Paetkau, 2003). The number of high-confidence loci (i.e., loci with two high-confidence allele calls) was then compared across all samples. Results for each sample were reported as number of loci with any genotype information and the number of loci with high-confidence genotype information.

Statistical analysis. We used Poisson count regression to test the relationship between the number of loci that were scored with high confidence and indices of environmental conditions that may have influenced the condition of DNA. We conducted two analyses, relating the total number of loci and the number of high-confidence loci to average temperature and month in which the fecal samples were collected. For month, we used deviation coding to contrast March, April, May, and June. Two samples collected on February 21 were coded as March.

We used Akaike's Information Criterion for small samples to identify the most parsimonious model (Anderson et al. 2000). To assess the predictive accuracy of the final models, we used a Wilcoxon signed-rank test to determine whether the distribution of observed loci differed from the number predicted by the count models. We used a jackknifing procedure to sequentially withhold each record during model fitting and then predicted the count for that withheld record. This allowed for an out-of-sample test, with sufficient data to fit the models (i.e., $n = 39$).

Results and Discussion

Very few of the fecal samples collected resulted in high-confidence genotype information. Of the 40 samples, only eight provided high-confidence data for more than one of the eight microsatellite markers (Table S1, *Supplemental Material*). Samples collected in March yielded the most complete genotype information, with an average of seven loci showing genotype information (four with high-confidence data). Genotyping success was much lower for samples collected during April from snow and bare ground, but not as low as those collected after April from bare ground (Figure 2).

The most parsimonious Poisson models representing the count of total loci and high-confidence loci consisted of the categorical variable month, representing the day of the year that the sample was collected (Table 1). The Wilcoxon signed-rank test revealed that the most predictive models were those constructed from the data representing the number of high-confidence loci. The number of loci (both those with some genotype information and those with high-confidence information) was positively correlated with earlier sample collection months (especially those at or below freezing temperatures with snow). That relationship decreased in strength through April and May and was strongly negative in June. Overall, genotyping success decreased for pellets collected after April (Table 2) when weather records indicate warmer and wetter conditions.

Because DNA degrades with time (Fernando et al. 2003; Piggot 2004), knowing when pellets were deposited and the amount of time that passed between deposition and collection would have helped us separate

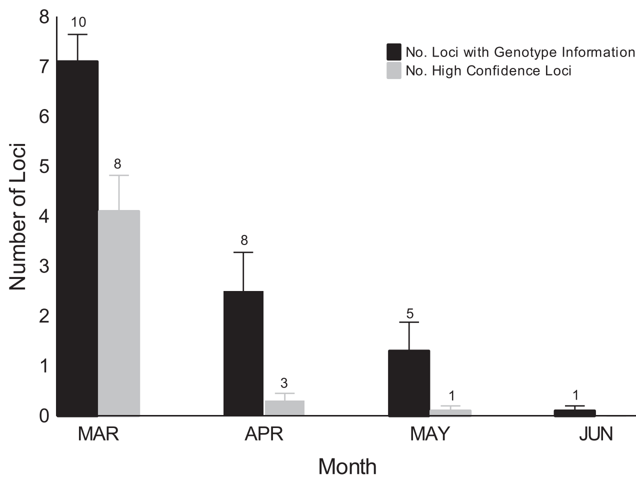

Figure 2. Mean (±1 SE) number of (eight possible) microsatellite loci obtained from fecal samples containing genotype information and of the number of loci containing high-confidence genotype information for both alleles (i.e., high-confidence loci) obtained from moose *Alces alces* fecal samples collected during mid-March, April, May, and June 2013 from the John Prince Research Forest in north central British Columbia, Canada. Note that numbers above the error bars indicate the number of samples from each month ($n = 10$ samples collected per month) that contained loci with some genotype information (above black bars) and with high-confidence loci (above gray bars).

the confounding effects of pellet age from collection season (Piggot 2004). Unfortunately, there was no way for us to age the pellets we collected. We are confident, however, that pellets were relatively recent (being deposited in those months before our collections and not from the previous summer or fall) based on their condition and content (Rea et al. 2010). Also, our objective was not to age pellets, but to assess genotyping success (a measure of fragment size, purity and/or template numbers) as a factor of warmer and wetter environmental conditions. Ultimately, genotyping success is directly related to the quality (usually measured in terms of state of DNA degradation [i.e.,

Table 1. Number of model parameters (k), differences in Akaike Information Criterion for small samples (AIC_c) scores (Δ), and AIC_c weights (w) for Poisson count models representing the high-confidence microsatellite loci and total number of loci with genotype information from moose *Alces alces* fecal pellets collected in north central British Columbia, Canada, from March to June 2013. Predictive ability of each model was tested through a comparison of observed and predicted number of loci using the Wilcoxon signed-rank test.

Model	k	$AIC_c\Delta$	AIC_cw	Wilcoxon P (z)
High-confidence loci				
Average temperature	2	8.9	0.012	0.010 (−2.590)
Month	4	0	0.988	0.312 (−1.012)
Total loci				
Average temperature	2	15.2	0.001	<0.001 (−4.887)
Month	4	0	0.999	<0.001 (−5.514)

Table 2. Model coefficients, standard errors (SE), and 95% confidence intervals for Poisson count models representing the high-confidence microsatellite loci and total number of loci with genotype information from moose *Alces alces* fecal pellets collected in north central British Columbia, Canada, from March to June 2013. Covariates represent two models, temperature and month, for each set of data.

Covariate	Coefficient	SE	95% Confidence interval	
High-confidence loci				
Temperature model				
Constant	0.085	0.248	−0.400	0.571
Average daily temperature	−0.164	0.018	−0.198	−0.129
Month model				
Constant	−4.705	0.281	−5.256	−4.155
March	6.116	0.305	5.518	6.714
April	3.502	0.445	2.629	4.375
May	2.403	0.735	0.962	3.843
June	−12.020	0.271	−12.550	−11.490
Total loci				
Temperature model				
Constant	1.227	0.131	0.971	1.483
Average daily temperature	−0.100	0.013	−0.126	−0.074
Month model				
Constant	0.219	0.273	−0.317	0.754
March	1.741	0.278	1.196	2.286
April	0.737	0.340	0.070	1.404
May	0.044	0.407	−0.755	0.842
June	−2.521	0.732	−3.957	−1.086

average fragment size] and purity [i.e., lack of inhibitors]) and quantity (i.e., concentration of intact templates) of the recovered DNA. However, whether changes in DNA fragment size, purity, or the number of template molecules present in feces were due to seasonal effects or pellet age or a combination of the two is irrelevant relative to our objectives. Pellets collected earlier in the spring provided higher genotyping success (Figure 2).

Although Piggot (2004) reported that DNA collected from summer-deposited scats of red fox *Vulpes vulpes* and brush-tailed rock wallaby *Petrogale penicillata* had better quality DNA than winter-deposited scats, Harris et al. (2010) suggested that Afghanistan Argali sheep *Ovis ammon* fecal materials yielded better quality DNA when collected in winter. Maudet et al. (2004) also obtained better DNA from Alpine ibex *Capra ibex* and Corsican mouflon *Ovis musimon* pellets collected during winter (November–February) compared to collections from the spring (April–May). Maudet et al. (2004) postulated that the low-quality, high-fiber diets in winter are more likely to increase the amount of time digesta spend in contact with the gastrointestinal tract, thereby allowing intestinal mucosal cells to accumulate on fecal materials and increase the quantity of DNA recovered per pellet.

Wehausen et al. (2004) suggested that pellets collected from herbivores foraging on plants high in secondary compounds may be hard to assess for fecal DNA because of inhibitory effects of plant secondary compounds on polymerase chain reactions (Kreader 1996). Plant secondary compounds consumed by moose eating new

shoots and leaves may have potentially influenced the purity of DNA from pellets we collected in June, but they would be negligible in the winter twigs eaten by moose in our study area from March to May before leaf flush (Palo 1984). Mucosal DNA collected from carnivores was also of higher quality when collected in winter compared to summer (Lucchini et al. 2002; Mowry et al. 2011), suggesting something other than plant secondary compounds (such as cooler conditions) may be influencing DNA recovery.

In summary, our findings suggest that if noninvasive collection of DNA is to be used as a tool to identify or track the movements of individual moose, fecal pellets should be collected during late winter and very early spring before temperatures warm. For many locations across the circumpolar range of moose, this is the time of year when winter-deposited pellets are being uncovered as snow melts, making sample collection relatively efficient. Our data suggest that pellets collected from snow or from bare ground shortly after snow melt are more likely to have DNA of sufficient quantity and quality for microsatellite genotyping than those pellets collected during the warmer spring and summer months. As such, pellets collected in late winter and early spring are more likely to help us understand the biology and ecology of moose (e.g., Ebert et al. 2012; Goode et al. 2014).

Supplemental Material

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Table S1. Genotype information for loci typed from moose *Alces alces* fecal pellets collected in March (MR), April (AP), May (MA), and June (JU) 2013 in the John Prince Research Forest, British Columbia, Canada. Sample identification is coded as month and sample number. Only samples with loci with genetic information (LGI) are shown. High-confidence loci calls are shown in bold. A summary of LGI and high-confidence loci (HCL: loci with two high-confidence allele calls) are shown for each sample.

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