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TECHNICAL NOTE

Individual identification of Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) using DNA from fecal pellets

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Abstract We tested a protocol for extracting DNA from fecal pellets from Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) and evaluated genotyping performance of previously developed microsatellite markers as well as a suite of new markers designed specifically for this study. We screened 30 microsatellites, and identified 7 (23%) loci including 4 new markers, that fit well into a single multiplex and consistently genotyped deer with low error rates. DNA was extracted from 2,408 fecal-pellet samples. Of those, 1,240 (52%) were genotyped successfully at all 7 markers allowing identification of 634 genetically unique deer. Using DNA from fecal pellets collected in the field was an effective technique for identifying and distinguishing among deer.

Keywords Alaska · DNA · Feces · Microsatellites · *Odocoileus hemionus sitkensis* · Sitka black-tailed deer

Densely vegetated environments within Southeast Alaska have hindered the collection of basic population parameters on Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), the most important terrestrial game species in this region. Because of challenges in sampling deer via direct observation (e.g., aerial surveys), we sought non-invasive methods using genetics (Waits and Paetkau 2005) to answer key population questions. We tested a protocol for extracting DNA from fecal pellets and evaluated genotyping performance of previously developed microsatellite markers as well as a new suite of markers designed specifically for Sitka black-tailed deer. Whereas several studies have been conducted on wild carnivores using non-invasive genetic approaches to identify individuals (Waits and Paetkau 2005; Kendall et al. 2008; Schwartz and Monfort 2008), field research identifying individuals using DNA from the feces of wild ungulates has been rare (Flagstad et al. 2000; Gebremedhin et al. 2009). Brinkman et al. (2009) demonstrated that deer feces collected from the rectum are a viable source of DNA. We now seek to assess the utility of fecal DNA from naturally deposited pellets to genotype deer (*Odocoileus* spp.).

During 2006–2008, we collected 4–6 fecal pellets from pellet groups deposited by deer in three watersheds on Prince of Wales Island in southeast Alaska. To minimize DNA degradation from ambient environmental conditions and to maximize DNA recovery, we collected pellets from transects that were cleared of old pellets 10 days earlier. We preserved pellets in 95% ethanol, and stored pellets at room temperature until DNA was extracted. During collection, pellet samples were classified based on appearance as: good (freshly deposited in a clumped distribution with pellets intact, surface with a glossy sheen, and/or a detectable coating of mucus), average (slightly older or more weathered pellet group that still had intact pellets with smooth surfaces, but that lacked a tightly clumped distribution, glossy sheen, and mucus), or poor (spread-out groups with rough-surfaced pellets which were often showing signs of decomposition). Because early experimentation revealed

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that "poor" samples consistently failed to amplify, we excluded them from further analysis.

We extracted genomic DNA from fecal pellets using the DNeasy Tissue Kit (Qiagen Inc. Valencia, CA), with slight modifications. During 2006 and 2007, we used the DNeasy Tissue Kit and a protocol described by Maudet et al. (2004) with the following modifications: we performed lysis of one fecal pellet in 25 ml scintillation vials on a rocker at room temperature for 20 min using 900 µl of lysis washing buffer. During 2008, we used the DNeasy Tissue Kit lysis buffer (ATL) instead of the lysis solution described by Maudet et al. (2004). Also during 2008, we placed two pellets in vials on a rocker for 1 h using 800 µl of Qiagen ATL lysis solution. We adjusted agitation during the pellet wash to thoroughly harvest intestinal mucosal cells from the exterior of the pellet without breaking apart the pellet.

We conducted PCR in 10-µl reaction volumes using Qiagen Multiplex Master Mix® (Qiagen, Valencia, CA) according to manufacturer's instructions. Our optimum thermal profile began with an initial 15-min 95°C denaturing step, followed by 45 cycles of 94°C (1 min), 61°C (1 min 30 s) and 72°C (1 min) followed by a 30-min extension at 60°C. We used an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) to determine genotypes.

Our error checking protocol included the "multi-tube" approach, where we analyzed DNA samples multiple times to ensure accuracy (Taberlet et al. 1996). We scored and checked markers manually using GeneMapper 3.7 software® (Applied Biosystems, Foster City, California). We used software Micro-Checker (van Oosterhout et al. 2004) to detect samples likely containing genotyping errors (scoring, stuttering, null alleles, and dropout). We excluded all monomorphic markers from identity analysis. We excluded polymorphic markers from identity analysis when errors (e.g., amplification failure, null alleles) per reaction exceeded 20%. For usable markers, we reported errors per reaction, summarized for each locus and over all loci. We assessed overall genotyping repeatability by re-amplifying and re-genotyping a subset (30%) of successfully genotyped samples. We calculated allelic diversity, probability of identity (PID), and PID for sibs (PIDSIB) using GenAlEx (Peakall and Smouse 2006). We sought PID values <0.001 and PIDSIB values <0.05 (Schwartz and Monfort 2008).

We screened 30 microsatellite primers for variability and suitability for use with DNA extracted from deer pellets. Seven of those primers were newly designed (Genetic Identification Services, Chatsworth, California) for this study (Table 1). Twenty-six markers (87%) amplified, 20 (67%) were variable, and 7 (23%) amplified consistently with error rates <20% and fit well into a single multiplex; thus, we included those 7 loci in analysis of individual identification (Tables 1, 2). Multiplex PCR reactions contained adjusted concentrations of each primer set to achieve optimum allelic peaks and minimize amplification noise and stuttering (Table 2). Using Micro-Checker, we found no evidence of excess scoring error due to stuttering and no evidence of frequent allele dropout among the 7 loci used in identity analysis. When all years were grouped, evidence for null alleles was present at locus T7 in one watershed and T159S in one watershed due to an excess of homozygotes. However, this problem was assumed to be minor because we found no evidence of null alleles at those loci in the other watersheds or when years were analyzed separately.

We extracted DNA from 2,408 fecal-pellet samples. At least 1 marker amplified PCR products from all samples, and 1,240 (52%) were genotyped successfully at all 7 markers. Genotyping success (consensus among replicated reactions) during 2008 (87%) was roughly double that of 2006 (41%) and 2007 (50%). Pooling all years, success rates of pellet samples classified as "good" (66%) was double that of pellets samples classified as "average" (33%) during collection. The highest amplification efficiency was 91%, observed in "good" pellets in 2008 after we altered our extraction method.

In total, we identified 634 genetically unique deer. Probability of identity for the population was 0.0003, and PIDSIB was 0.021 (Table 2). Of 382 samples re-amplified for error checking, errors were found in 10.2% of multiplex reactions. Summarized by individual loci, error rates per reaction did not exceed 5% (Table 2). Nine reactions (2.3%) failed to amplify at $\geq$1 locus, and amplification failure rate by individual locus did not exceed 1%, varying between 0.2 and 0.8%.

We determined that using DNA extracted from fecal pellets collected in the field was an effective technique for identifying individual Sitka black-tailed deer. Our findings suggest that field investigations of ungulate population parameters may be possible using fecal DNA without reference data. While only 23% of the microsatellites screened were determined to be adequate for inclusion in analysis of individual identification, those 7 markers worked well in a single multiplex reaction and our error rates (10%) rival other non-invasive studies (Hedmark et al. 2004 [12%]; Pilot et al. 2007 [16%]). Despite low levels of polymorphism, we were able to achieve an acceptable probability of identity (Schwartz and Monfort 2008). Adding a locus for gender determination of Sitka black-tailed deer (Brinkman and Hundertmark 2009) would increase the discriminatory power of our suite of loci by up to 2×, depending on the sex ratio.

By the final year of our study, our genotyping success (87%) was comparable to other non-invasive wildlife investigations (Belant et al. 2007 [75%]; Hedmark et al. 2004 [65%]; Kendall et al. 2008 [74%]) and likely was influenced by extraction protocol and condition of fecal pellet collected. Clearly, differences in genotyping success

Table 1 Description and performance of 30 microsatellite loci screened for use to identify individual Sitka black-tailed deer (SBTD) using DNA from fecal pellets

Locus	GenBank accession no.	Used for individual identification	*N*	Amplify?	Variable in SBTD?	Size (bp)	Number of alleles
C89[a]	AF102247	Y	2,408	Y	Y	161–169	2
SBTD06[j]	FJ986212	Y	2,408	Y	Y	176–188	3
SBTD04[j]	FJ986215	Y	2,408	Y	Y	238–302	8
SBTD05[j]	FJ986216	Y	2,408	Y	Y	110–130	3
SBTD07[j]	FJ986214	Y	2,408	Y	Y	177–197	5
T159S[a]	AF102245	Y	2,408	Y	Y	195–211	4
T7[a]	AF102240	Y	2,408	Y	Y	219–227	2
C106[a]	AF102243	N	2,032	Y	Y	289–297	3
T27[a]	AF102244	N	2,032	Y	Y	275–287	4
RT7[b]	U90740	N	784	Y	Y	209–217	2
BL42[d]	EU009439	N	784	Y	Y	244–250	2
BM1225[d]	G18419	N	784	Y	Y	230–232	2
C217[a]	AF102242	N	784	Y	Y	192–204	2
C273[a]	AF102246	N	784	Y	Y	144–172	2
RT24[b]	U90746	N	784	Y	Y	218–234	3
SBTD02[j]	FJ986211	N	784	Y	Y	142–150	2
SBTD01[j]	FJ986210	N	784	Y	Y	121–157	2
SR-CRSP-1[e]	L22192.1	N	784	Y	Y	141–143	2
T32[a]	AF102241	N	784	Y	Y	275–283	3
Texan4[f]	L24781	N	784	Y	Y	134–136	2
BM 4107[d]	G18519	N	10	Y	N	161	NA
IDVGA55 [g]	X85071	N	10	Y	N	181	NA
INRA121[h]	X71545	N	10	Y	N	205	NA
RT5[b]	U90738	N	10	Y	N	160	NA
SBTD03[j]	FJ986213	N	96	Y	N	243	NA
VH 110 (OarVH110)[i]	NW_001494486	N	10	Y	N	270	NA
BM 203[d]	G18500	N	10	N	N	NA	NA
BM 757[d]	G18473	N	10	N	N	NA	NA
TGLA53[c]	DS490633.1	N	10	N	N	NA	NA

[a] Levine et al. (2000)
[b] Wilson et al. (1997)
[c] Bonnet et al. (2002)
[d] Bishop et al. (1994)
[e] Arevalo et al. (1994)
[f] Holder et al. (1994)
[g] Mezzelani et al. (1995)
[h] Vaiman et al. (1994)
[i] Hanrahan et al. (1993)
[j] This study

between pellet groups classified as "good" (66%) and "average" (33%) illustrates that DNA quality can be assessed in the field.

Successful individual identification of Sitka black-tailed deer using DNA from fecal pellets provides wildlife managers with a new tool to monitor populations. Specifically, this technique may enable mark-recapture studies that can estimate key population parameters such as abundance and survival. This opportunity is particularly valuable because reliable estimates of population size for Sitka black-tailed deer have never been available. In addition, DNA-based identification from fecal pellets

Table 2 Description of the seven variable microsatellites used in multiplex PCR reactions for individual identification of Sitka black-tailed deer

Locus[a]	Primer dye color	Forward (μl)	Reverse (μl)	Sample size	PI	PI_{SIB}	Mean error rate (SE) per reaction ($n = 382$)
C89	NED™ (yellow)	5	5	634	0.672	0.822	0.010 (0.005)
SBTD06	6-FAM™ (blue)	10	10	634	0.376	0.596	0.026 (0.010)
SBTD04	6-FAM™ (blue)	15	15	634	0.156	0.459	0.042 (0.012)
SBTD05	NED™ (yellow)	10	10	634	0.369	0.591	0.021 (0.007)
SBTD07	VIC® (green)	10	10	634	0.305	0.555	0.045 (0.012)
T159S	PET® (red)	15	15	634	0.192	0.471	0.050 (0.012)
T7	NED™ (yellow)	15	15	634	0.384	0.605	0.008 (0.005)
All loci[b]	NA	80	80	634	0.0003	0.021	0.102 (0.015)

PID is the probability of identity assuming random individuals, and PID_{SIB} is the probability of identity assuming siblings. Mean error rate per reaction includes false alleles and dropouts

[a] Initial primer concentration = (2 μM)

[b] Primer mix = 160 μl primer with 340 μl TE buffer

potentially will allow researchers to advance understanding of social structure, paternity, kinship, sex ratios, gene flow and phylogeography, all of which are poorly understood for Sitka black-tailed deer.

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