

TECHNICAL NOTE

Shed skin as a source of DNA for genotyping seals

B. J. SWANSON,* B. P. KELLY,† C. K. MADDOX* and J. R. MORAN†

*Applied Technology in Conservation Genetics Laboratory, Brooks Hall, Central Michigan University, Mount Pleasant, MI 48859, USA, †School of Arts and Sciences, University of Alaska Southeast, Juneau, AK 99801, USA

Abstract

Obtaining a sufficient number of DNA samples from ice-breeding marine phocids, in a noninvasive manner, has proven difficult and has limited the ability to use molecular genetics on these species. We evaluate the ability to genotype ringed seals using a novel source of DNA, skin cells shed by the seal as it moults on sea ice. We found that shed skin samples yielded a lower quantity and purity of DNA compared to tissue samples. Nevertheless, the shed skin cells were a viable source of DNA for microsatellite analysis; we found no significant difference in allelic diversity or heterozygosities between tissue samples and shed skin cells. This source of DNA should allow the rapid collection of a large number of noninvasively collected DNA samples in ice-breeding phocids.

Keywords: arctic, genotyping, microsatellites, noninvasive, *Phocidae*, seals

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The introduction of polymerase chain reaction (PCR) prompted a rapid expansion in the types of samples from which analysable quantities of DNA can be obtained (Pääbo 1990), often called low copy DNA (lcDNA) sources (e.g. Gerloff *et al.* 1995; Ernest *et al.* 2002; Lucchini *et al.* 2002; Palomares *et al.* 2002). The ability to obtain usable quantities of DNA from sources such as faeces (e.g. Høss *et al.* 1992; Maudet *et al.* 2004), blood (e.g. Dallas *et al.* 2003; Scandura 2005), hair (e.g. Goossens *et al.* 2000; Constable *et al.* 2001), urine (e.g. Hedmark *et al.* 2004), feathers (e.g. Rudnick *et al.* 2005) and scales (e.g. Miller & Kapuscinski 1997; Säisä *et al.* 2003) has also revolutionized data collection methods for biologists. Non-invasive DNA collection is now commonplace, reducing danger and stress to both animals and biologists and increasing the ability to obtain sample sizes necessary for accurate parameter estimation (Taberlet *et al.* 1997; Creel *et al.* 2003).

The increasing frequency of lcDNA use also led to the recognition of several problems associated with these sources such as allelic dropout or misprinting; errors which can occur at high frequencies (Creel *et al.* 2003). Allelic dropout and misprinting are associated with lcDNA due to the low number of cells found in most samples and the extensive exposure to environmental

conditions often associated with the collection of lcDNA. Low-copy DNA samples typically are removed from the environment after exposure to ambient conditions for hours to days or even longer. Depending upon the season, the samples can be exposed to intense periods of UV radiation, temperature fluctuations and precipitation, resulting in degraded DNA. Several approaches to reducing the impact of these errors have been developed (e.g. Taberlet *et al.* 1996; Morin *et al.* 2001; Miller *et al.* 2002; Creel *et al.* 2003; Bellemain & Taberlet 2004).

Perhaps the most valuable impact of noninvasive sampling has been the increased ability to sample rare or elusive species (Taberlet *et al.* 1997; Ernest *et al.* 2000; Hedmark *et al.* 2004; Swanson & Rusz 2006). These techniques, however, have been of limited utility in sampling marine mammals, even if the species of interest spend some portion of their time on land or ice (but see Reed *et al.* 1997; Parsons *et al.* 1999; Parsons 2001).

Most species of seals spend the majority of their time in the ocean, coming out of the water to molt, rest, give birth and nurse their young. Sampling ice dwelling mammals, such as ringed seals (*Phoca hispida*), can be even more difficult as they rarely are found far from accessible water. Nursing phocids rapidly put on weight minimizing the amount of time they, and their mothers, spend on the ice (Perry *et al.* 1995). Thus, obtaining a sufficient number of samples from marine mammals in a nondestructive and noninvasive manner is problematic.

Correspondence: Bradley J. Swanson, Fax: 989 774 3462; E-mail: brad.swanson@cmich.edu

Table 1 Observed heterozygosity (H_o) and the number of alleles (A) found at each locus. Samples came from either live-trapped seals (tissue) or from skin cells collected around breathing holes (skin). P value refer to the comparison between heterozygosity values and allelic diversity values for the skin samples vs. the tissue samples. All P values are based on 1000 resamplings of the data for the tissue samples down to the sample size for the skin samples

	SGPV9		SGPV10		SGPV16		Hg4.2		Hg6.3		Hg8.10	
	H_o	A	H_o	A	H_o	A	H_o	A	H_o	A	H_o	A
Total	0.67	11	0.87	17	0.83	11	0.84	18	0.88	17	0.51	15
Skin ($n = 18$)	0.56	7	0.80	13	0.78	7	0.81	10	0.94	10	0.56	8
Tissue ($n = 51$)	0.69	9	0.90	16	0.83	11	0.89	17	0.87	17	0.67	14
P value	0.27	0.84	0.36	0.78	0.33	0.62	0.41	0.20	0.83	0.17	0.58	0.19

In May 2005, shed skin samples were collected from the ice around ringed seal breathing holes in the shorefast ice of the Chukchi Sea. Subnivean breathing holes were located by Labrador retriever dogs trained to dig in the snow where they detected the odour of ringed seals (Smith & Stirling 1975; Kelly & Quakenbush 1987). Other sample sites were located by visually scanning the ice for seals resting on the surface of the ice. Additional skin samples were collected from ringed seals captured at their breathing holes using the methods described by Kelly (1996). A 5 mm × 5 mm skin sample was clipped from the webbing of the hind flipper of each captured seal, placed in a coin envelope, and stored dry at approximately 10 °C.

Individual flakes of shed skin were collected from the surface of the ice next to breathing holes using sterilized forceps, placed in to a coin envelope, and stored dry. The location of each hole was recorded using hand-held GPS receiver along with the date, time and a description of each site including evidence that more than one seal had been present.

Extractions were performed with QIAGEN DNeasy kits following published protocols (QIAGEN 2001) and DNA quantity and purity were measured with an Eppendorf BioPhotometer (Brinkman Instruments Inc.) using single use uvets (Fisher Scientific). Samples were diluted to a working stock of 15 ng/μL, and any remaining tissue and extracted DNA were stored at -80 °C. All seals were genotyped at six microsatellite loci developed for harbour seals (Allen *et al.* 1995; Goodman 1998). All reverse primers were fluorescently labelled with FAM, TET, or HEX. PCR was performed in a 20-μL cocktail containing 75 ng genomic DNA, 250 μM dNTPs, 0.16 μM of each primer, 1× HotMaster *Taq* buffer, and 1.5 U of HotMaster *Taq* polymerase (Brinkman Instruments Inc.). Amplification was conducted on an Eppendorf MasterGradient Thermocycler (Brinkman Instruments Inc.) and consisted of initial denaturation step for 2 min at 94 °C followed by three cycles of 20 s at 94 °C, 20 s at 52 °C, and 5 s at 72 °C. This was followed by 33 cycles of 15 s at 94 °C, 20 s at 52 °C, and

5 s at 72 °C, followed by a terminal extension step of 1 min at 72 °C. Following amplification, samples were analysed on an ABI PRISM 310 Genetic Analyser using GENESCAN ANALYSIS 3.1.2 software (Applied Biosystems). Genotypes were determined using GENOTYPER 2.0 software (Applied Biosystems).

All shed skin samples, which produced a homozygous genotype, were re-run in two additional PCRs to minimize the possibility of allelic dropout. In cases where any of the three PCRs indicated a heterozygote existed, the individual was classified as a heterozygote.

We found that the tissue samples (mean = 105.6 ng/μL, SE = 8.0) had a significantly higher DNA concentration ($t_{df=97} = 8.2$, $P << 0.0001$) than the shed skin samples (mean = 29.4 ng/μL, SE = 4.8). The tissue samples (mean = 1.6, SE = 0.02) also had a significantly higher ($t_{df=97} = 10.0$, $P << 0.0001$) 260/280 absorbance value than the shed skin samples (mean = 1.1, SE = 0.05). A pure DNA preparation should produce a 260/280 purity value of 1.8 (Manchester 1995).

We were unable to achieve any amplification for 5 (15.6%) of our shed skin samples. It was possible that a shed skin sample could contain DNA from more than one individual given our collection methods; we classified a sample as containing multiple individuals if any locus showed more than two alleles in any amplification at any locus. We found evidence of multiple seals within a sample in nine of the 27 samples (33%). Notably, eight of these samples were identified in the field as likely containing multiple seals. All of the samples containing multiple individuals were removed from all further analyses. GENEPop indicated that none of the loci in either the tissue or the shed skin samples were out of Hardy–Weinberg equilibrium (all $P > 0.18$) and none of the loci showed linkage disequilibrium (all $P > 0.22$). Allelic drop out was rare in the shed skin samples and was found in only five of 216 (2.3%) amplifications.

We found high levels of heterozygosities at all loci (0.59–0.88; Table 1) and a large number of alleles at all loci (11–19; Table 1). We found no significant difference (all

$P > 0.27$) in the average heterozygosities at any of the loci based on sample type following 1000 resamplings to equalize sample size (Table 1). We found the same pattern with respect to allelic diversity based on 1000 resamplings; there were no significant differences (all $P > 0.17$) in the number of alleles at any of the six loci based on the source of the DNA (Table 1).

Despite the harsh conditions the shed skin cells were exposed to while on the ice, they still provided a valuable and reliable source of DNA. All of our noninvasively collected samples produced either complete genotypes or no amplification, and we found a very low level of allelic dropout. We conclude that shed skin samples can be used to accurately characterize an individual. Similarly, the skin cells shed on the ice next to the seals' breathing holes had the same average heterozygosity and allelic diversity as the DNA from more traditional sources indicating that they can be used to characterize a population as well.

The ability to use shed skin cells from marine mammals will improve the ability of researchers to obtain a large number of genetic samples on species which, previously, were difficult to sample in sufficient quantity. This should positively impact biologists' ability to investigate the breeding structure of pinnipeds and improve our ability to manage these species as well.

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