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A technique for sampling ancient DNA that minimizes damage to museum specimens

S.M. Wisely*, J.E. Maldonado & R.C. Fleischer
Genetics Program, Smithsonian Institution, 3001 Connecticut Ave. NW, Washington, DC 20008, USA
*(*Corresponding author: Phone: (202) 673-4781; Fax: (202) 673-4648; E-mail: wisely.samantha@nmnh.si.edu)*

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Because of the utility of ancient DNA to conservation genetics (Baker 1994), the number of requests to collect tissue from museum specimens has increased. A drawback of consumptive sampling is that it requires removal and destruction of part of the specimen. Epithelium, hair, dried skeletal muscle, and bone have been used as sources of ancient DNA taken from skulls, postcranial material or study skins (Herrmann and Hummel 1994). Although many museums permit consumptive sampling of collections, the request is generally contrary to the goal of collection managers, which is long-term care and maintenance of specimens. We propose a collection method that attempts to satisfy goals of both loan requestor and collection manager, the use of maxilloturbinal bone material. Maxilloturbinates are thin bones attached anteriorly to ridges inside the nasal cavity (Hillenius 1992). In this study we compare success rates in amplification of genomic DNA taken from epithelium and maxilloturbinates ranging in age from 17 to 111 years old.

We sampled 5–20 mg epithelial or 10–20 mg maxilloturbinal bone tissue of black-footed ferrets (*Mustela nigripes*) from six collections. Curatorial staff collected epithelial tissue from study skins. We instructed staff to sterilize collection tools before and in between handling specimens using 50% bleach solution and to deposit samples in sterile containers. Epithelial samples included material from the ventral incision of study skins and clippings from the inner ear. All maxilloturbinal samples were collected by one author (SMW). Forceps sterilized with 50% bleach were inserted into the nasal cavity of the skull, and bone material was carefully dislodged. No force was applied to retrieve 20 mg of material. Fragments were collected onto sterile aluminum foil and poured into a sterile 2 ml screw cap tube.

We extracted and amplified DNA from epithelial and turbinal samples in an isolated ancient DNA laboratory. Epithelium was cut into small pieces; turbinates were already fragmentary. Samples were incubated at 58 °C for 24 hrs in lysis buffer (10 mM Tris, 2 mM EDTA, 10 mM NaCl, 1% SDS, 10 mg/ml DTT and 10 mg/ml protienase K), extracted with phenol twice and chloroform once. Three ml of deionized water were added to the final supernate and cleaned with a Centricon 30 dialysis filter (Amicon). Deionized sterile water was added so that the final volume was 200 μl. The extracted DNA solution was aliquotted and stored at –20 °C.

We amplified nuclear DNA using eight microsatellite loci [Mvis075, Mer049, Mvis002 (Fleming et al. 1999), Mer095 (Wisely et al. 2002), Mvi57, Mvi87, Mvi232 (O'Connell et al. 1994) and G1A (Davis and Strobeck 1998)]. Allele sizes of loci ranged from 104 to 227 bp. Plasticware was UV treated and all surfaces wiped with 50% bleach prior to lab work. DNA was amplified using the following polymerase chain reaction (PCR) conditions: 1X Sigma Gold buffer without $MgCl_2$, 2.5mM $MgCl_2$, 2 mM dNTP's, 2.1 mg/ml BSA, 0.8 m betaine, 1 μM each primer, 1U Taq Gold (hot start), cycled 45 times at 92 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min. Conditions were optimized using epithelial samples. PCR was performed twice per genotype and products were visualized using a 373 ABI automated sequencer.

We compared number of genotypes amplified in epithelial vs. turbinal samples using a Wilcoxon signed rank test. To test for effect of specimen age on amplification success, we regressed number

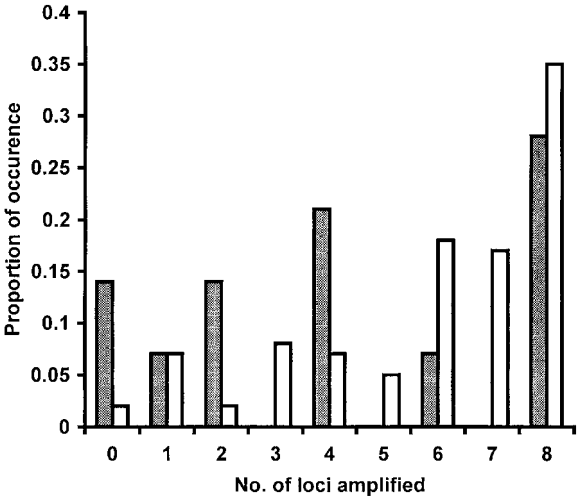

Figure 1. The proportion of occurrence for the number of loci amplified using DNA extracted from epithelial tissue (solid) or turbinal tissue.

of genotypes amplified onto year in which the museum specimen was collected (SPSS V. 10.0). We considered allelic dropout to occur when replicate amplifications yielded genotypes that changed from homozygote to heterozygote or vice versa. We tested for differences in allelic dropout between tissue types using contingency table analysis. We tested for correlation in allelic dropout and collection year using logistic regression. Although estimates of allelic dropout from one replicate likely underestimate the absolute rate, the estimates provide a basis for comparison between techniques.

We collected 14 epithelial samples and 62 turbinal samples from 76 museum specimens; each sample, epithelial or turbinal, was from a unique individual. From turbinal samples we obtained analyzable PCR product for 73% of 496 possible genotypes (8 loci × 62 samples). In contrast, only 42% of 112 possible genotypes from epithelial tissue amplified. We amplified significantly more genotypes per individual from turbinates (5.9 ± 0.3, mean ± SE) than from epithelium (4.1 ± 0.6, $Z = -2.0$, $P = 0.05$, Figure 1). We found no correlation between number of loci amplified and collection year for turbinates ($r^2 = 0.008$, $F_{1,61} = 0.50$, $P = 0.50$) or for epithelium ($r^2 = 0.45$, $F_{1,13} = 3.05$, $P = 0.10$). We found no difference in frequency of allelic dropout in genotypes amplified from epithelium (26.9%) vs. turbinates (26.5%, $\chi^2 = 0.002$, $P = 0.96$), nor was dropout frequency correlated with collection year ($\chi^2 = 2.11$, $P = 0.15$).

DNA in bone comes from osteoblasts, which are cells protected from air, humidity and UV light by the calcium matrix they form. It is not surprising that turbinal bone preserved DNA better than epithelial tissue. DNA extractions from turbinates amplified more genotypes than epithelial tissue, although age of the specimen did not affect amplification success in turbinal or epithelium samples.

In this study we demonstrate the utility of turbinates as a source for ancient nuclear DNA; Hoelzel et al. (2002) successfully used turbinates as a source of mitochondrial DNA. Consumptive sampling techniques that use bone or teeth require drilling or cutting bone fragments, which can destroy morphological landmarks valuable to taxonomic studies. A literature search yielded no studies that used maxilloturbinates of museum specimens. We believe that consumptive sampling of maxilloturbinates does not compromise the scientific utility of a museum specimen for morphometric or character studies, yet provides geneticists with well preserved DNA for conservation genetic studies. Nonetheless, we recommend that one side of the nasal cavity be left intact and at least one voucher specimen be left unsampled in collections to ensure that unforeseen morphological research is not hampered. We recommend that this technique only be used in mammals larger than 100–300 g, where turbinates can be easily accessed without damaging the skull.

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