



Weathered Antlers as a Source of DNA

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ABSTRACT We tested antlers of Coues white-tailed (*Odocoileus virginianus couesi*) and mule deer (*O. hemionus*) in various stages of natural decomposition to determine the degree of weathering that cast antlers could endure and still yield usable DNA. Based on physical characteristics, we partitioned antlers into 7 weathering categories ranging from freshly cast (class 1) to having been exposed to weathering for ≥ 8 years (class 7). Most antlers that had weathered 1–7 years yielded amplifiable DNA, whereas antlers weathered for ≥ 8 years failed to yield replicable DNA. This study confirms that antlers exposed to weathering for 1–7 years can be a source of replicable DNA and, thus, contribute an additional, and previously overlooked, non-invasive sample for analysis in molecular genetics studies. Published 2012. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS antlers, Coues white-tailed deer, DNA, mule deer, *Odocoileus hemionus*, *Odocoileus virginianus couesi*, Southwestern United States, weathering.

Population genetic studies rely mainly on tissues collected from living or freshly killed specimens. After an organism dies, its DNA normally degrades due to activity of endogenous nucleases, enzymes that cleave nucleic acids and break apart the DNA molecule. Nuclease enzymes can be destroyed or inactivated by rapid desiccation, low temperatures, or high salt concentrations, so that DNA may be preserved by these processes (Hofreiter et al. 2001). DNA extraction and sequencing has been performed on ancient bone samples ranging from 4 years old to $>50,000$ years old (Höss et al. 1996). For specimens collected under controlled conditions and stored in museums for up to 200 years, retrieval of DNA sequences using polymerase chain reaction has become routine (Hofreiter et al. 2001). Soon after collection, such samples are usually protected from environmental factors (heat, moisture, sunlight) that would degrade DNA. Unlike soft tissue, which degrades rapidly after an organism dies, antlers offer a potentially useful source of DNA for genetic studies.

Environmental conditions affect weathering rates of antler and bone and, perhaps, their utility as DNA sources. Tappen (1994) concluded that weathering and crack formation on bones was slower in a rainforest environment than in open savannas of Zaire. Behrensmeyer (1978) concluded that exposure to sunlight and rain, and extreme fluctuations in these factors, increased weathering rate of individual bones in Kenya. In this study, we investigated whether antlers with varying degrees of natural weathering can be used as an alternate source of DNA, as a substitute for blood, hide, and flesh or muscle tissue collections, which generally require either capture of a live animal or finding of a deceased one.

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STUDY AREA

Collectors provided deer antlers that had been gathered approximately between 1993 and 2002 in southwestern New Mexico and central and southeastern Arizona (USA). Typically, cast antlers are found in areas with the highest densities of deer; for Coues white-tailed deer (*Odocoileus virginianus couesi*), these areas were the isolated sky-islands of southeastern Arizona and along the Mogollon Rim, primarily in mixed pine–oak (*Pinus* spp.–*Quercus* spp.) woodlands and higher elevation semi-desert grasslands (Hoffmeister 1962, Knipe 1977).

Habitats where mule (*O. hemionus*) and white-tailed deer antlers were collected characteristically received a yearly average of 42 cm of precipitation, of which >15 cm could be expected to fall during the summer months. Precipitation ranged from 43 cm near Kingston, New Mexico at the eastern end of the study area, to 53 cm south of Flagstaff, Arizona along the Mogollon rim, and to 35 cm in the Huachuca and Dos Cabezas mountains of southern Arizona.

METHODS

Available Coues white-tailed deer and mule deer antlers were tested to determine the amount of natural weathering antlers could endure and still yield DNA. We tested 112 antlers: 76 individual Coues white-tailed deer and 13 individual mule deer antlers; and 23 antlers from either species that were still attached to skull plates. Of the combined total 135 samples, 112 shed antler and 23 attached to skull plates, only three were from museums with the remainder coming from private collections.

We extracted a small amount of antler material by driving an 8-mm drill bit into the base of the antler (where antler attaches to pedicle bone). Shavings retrieved from the initial 1–2 mm of the drill hole were discarded (O'Connell and Denome 1999). We collected antler material by drilling to a

depth of 2–3 cm and collecting material on a clean sheet of paper. We then transferred antler material to individually labeled 2-ml vials. The drill bit was flame-sterilized between each sample (O'Connell and Denome 1999). Drill speed was kept at low revolutions per minute to avoid scorching the antler shavings. For antlers attached to skulls, we drilled into the base of the antler through the skull plate toward the pedicle bone and discarded the skull bone. In 4 cases where the owner of a skull was unwilling to allow the antler to be drilled, a sample was extracted directly from the pedicle bone rather than the antler.

We sent antler shavings to Wildlife Genetics International (WGI; Nelson, BC, Canada) for DNA extraction and individual genotyping. Genotyping was performed using a suite of 12 microsatellite genetic markers that WGI uses for white-tailed deer.

Micro-Checker, an application designed to check microsatellite data for null alleles and scoring errors, was used for each weathering class. A logistic regression using replicable DNA as the response, with source as the predictor, was used to compare antler and pedicle bone shavings.

Weathering categories of antlers were based on the physical appearance of each antler. Collectors were asked to assign antlers into 7 weathering classes. Although assignment criteria are qualitative and assignment is highly subjective, weathering classes 1–6 approximately correspond to years of field exposure, and class 7 corresponds to ≥ 8 years of field exposure. Field exposure is defined as the number of years an antler was on the ground exposed to the elements before being collected.

RESULTS

Ninety percent of antlers in weathering classes 1–6 yielded useable DNA. Antlers in weathering class 7 failed to yield DNA (Table 1). These weathered antlers had characteristic large cracks along the entire length of the antlers, brittle and/or crumbly exterior, and pinkish color (probably caused by bacterial growth; B. Yates, United States Fish and Wildlife Service Forensics Lab, personal communication). One set of antlers that had been on top of a barn for 8–10 years in southern Arizona had pedicle-bone shaving collected twice in an attempt to extract DNA. Both attempts failed to produce usable DNA; the material was degraded beyond use.

Evidence for the presence of null alleles at several loci was detected across most weathering classes. Antlers in weather-

ing class 1 had significant evidence for null alleles at 2 loci. Antlers in weathering class 2 had significant evidence for null alleles at 4 loci. Weathering class 3 had significant evidence for null alleles at 3 loci. Weathering class 4 had significant evidence at 1 locus for null alleles. Finally, weathering classes 5 and 6 (analyzed together due to their small samples size) did not show evidence for null alleles. Given these results, there was no apparent pattern in the abundance of null alleles.

In our analysis, antlers exposed to weathering for 1–4 years yielded amplifiable DNA for 93% of all attempts, whereas samples with >7 years of field exposure failed to produce usable DNA (Table 1). Results obtained from antler shavings versus pedicle bone shaving did not differ ($P = 0.576$).

DISCUSSION AND MANAGEMENT IMPLICATIONS

Cast antlers, as a potential source of DNA, are easily obtained and relatively abundant. They are collected by many people for a variety of reasons, including sale for profit and for display. Many individuals who collect antlers record the date and location where antlers were found. Specimens from such collections could be an important source of genetic material.

Antler shavings are easy to store without spoilage for DNA extraction. This allows the use of DNA for analysis in a variety of studies including population differentiation (Balloux and Lugon-Moulin 2002) kinship, paternity, individual identification (Taberlet and Luikart 1999), population's effective size, and demographics (Luikart et al. 1998).

Gnawing of antlers by rodents can influence weathering of antlers by exposing the core, and gnawing can also decrease the presence of antlers by consumption. Most antlers exposed to the environment for >1 year showed evidence of gnawing, which is not an uncommon occurrence (Michael 1965). However, based on our results, gnawing did not appear to adversely affect antlers <6 years old in terms of their potential as a source of DNA. Rodents gnaw antler and bones, probably to satisfy mineral intake requirements or to maintain continuously growing, open-rooted incisors (Poole 1940).

This study confirms that antlers exposed to weathering for up to 7 years can provide replicable DNA and thus can contribute as additional, and often overlooked, non-invasive samples for analysis in molecular genetics studies. Most of

Table 1. Weathering class and number of loci amplified for Coues white-tailed and mule deer antlers gathered approximately between 1993 and 2002 in New Mexico and Arizona, USA.

Weathering class	No. samples	No. successful (approx. 10–12 loci)	No. moderately successful (approx. 5–9 loci)	No. failed (0–4 loci)	Overall success (%)
1	33	30	1	2	91
2	31	28		3	90
3	19	19			100
4	13	12		1	92
5	7	3	1	3	43
6	4	4			100
7	5			5	0
Total	112	96	2	14	86

our samples (96 of 107) in weathering classes 1–6 were fully successful in yielding replicable DNA (Table 1). Only 6 samples of antler shavings in weathering classes 1–4 failed to yield DNA. This might be attributable to scorching during extraction due to drill speed being too high, although there may be other possible explanations for failure to generate genotypes. All antlers in weathering class 7 (≥ 8 yr in the field) failed to produce replicable DNA; 7 years can, therefore, be interpreted as the threshold beyond which DNA is degraded in white-tailed and mule deer antlers collected in the Southwest. However, failures in weathering class 7 might be the result of the small sample size ($n = 5$) or other undetermined causes.

Our interpretation is that collected antlers that have been in the field up to 6–7 years are likely to yield useable DNA. Collectors should be able to identify antlers older than 7 years by their characteristic white “chalky” or pinkish stain and a crumbly surface, deteriorated tine tips, and large cracks along the main beam.

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