Correspondence

Ancient mitochondrial DNA from hair

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The DNA content of hair [1,2] is typically low compared to other tissues, as hair cells undergo dehydration and catabolic breakdown of nucleic acids and organelles during keratinisation [3]. As a consequence, ancient hair specimens have not been widely used as a source of ancient DNA. However, mitochondrial DNA (mtDNA) has been extracted from degraded and old hair samples, including burnt specimens [4], 100-year-old Native American samples [5], and wool from a 9,400 year old Bighorn sheep [6]. We have investigated the potential of hair as an aDNA source by analyzing DNA survival in 12 samples which range from 60 to >64,800 years of age and their susceptibility to contamination with modern DNA.

mtDNA was successfully amplified, cloned, and sequenced from 10 of the 12 hair samples following decontamination procedures (Table 1). DNA was quantified using Quantitative Real-Time PCR in a subset of the samples (Table 1). The survival of high copy numbers of 16S DNA from the 3,000 year-old Pazyryk horse hairs is consistent with the observation that DNA survives longer at sub-zero temperatures [7]. Of greater surprise was the persistence of high numbers of 16S and Control Region DNA molecules in hairs sampled from a bison mummy ^{14}C dated to >64,800 years. This result was independently replicated and extends the time frame from which authentic DNA has been retrieved from hair by at least seven-fold, placing it on a par with the oldest authentic DNA retrieved from bones and teeth [8]. No nuclear DNA could be amplified from the bison hair, consistent with observations of modern hair samples [1,9]. It is probably significant that the bison hairs are exceedingly well preserved — the atomic carbon to nitrogen ratio (3.47) is similar to modern mammal hair [10,11] and histological analysis of the specimen demonstrates the only structural modifications to be slight cuticular loss and adherent deposits (Supplemental data).

Amplifiable mtDNA was also present in all except two hair samples preserved at warmer temperatures, although at lower levels than the animal hairs. Several studies comment on the negative effect of various hair treatments (i.e., coloring/bleaching) on DNA survival [5,12,13], and it is possible that these two samples were artificially treated *pre* or *post mortem*. Hypervariable Region 1 (HVR1) sequences obtained from hair of Andaman Island specimens are consistent with previous dental studies [14]; in contrast, several hair samples attributed to Sir Isaac Newton each yielded different HVR1 sequences. The cloned PCR products indicated that each source of hair contained only a single HVR1 sequence, but one that did not match sequences from the other samples. As each hair source has been handled on numerous occasions, it seems unlikely that only a single contaminant sequence would survive per hair sample. However, we naturally cannot remove all reasonable doubts that they may still represent DNA contamination. We therefore suggest that at least three of the hair samples do not originate from Sir Isaac Newton. This hypothesis is in agreement with the conclusions of separate isotope analyses performed on the samples (A. Wilson unpublished data).

Many ancient specimens used for DNA analyses are difficult, if not impossible, to decontaminate from exogenous sources of DNA [8]. Surprisingly, although the DNA extracted from each animal hair sample was tested for contaminant human HVR1 sequences, none were detected. A similar lack of contamination appeared to apply to the human hair samples; no sequence variation was observed among cloned sequences (bar that which could be attributed to *post mortem* DNA damage [15]), even though the specimens have been handled multiple times by multiple individuals during their conservation history. These results suggest that hairs are either impermeable to sources of contaminant DNA (e.g., human sweat), or can be easily decontaminated (*e.g.* by bleach) to remove exogenous DNA. It is possible that this behavior is due to the hydrophobic nature of keratin, which comprises most of the hair shaft. The low quantities of free water associated with the keratin-packed hair cells may also reduce hydrolytic damage of the DNA. Such a scenario would explain the extended and high concentrations of DNA surviving in the bison and horse hair, as well as the relatively low levels of hydrolytic damage-induced lesions among the cloned DNA.

The successful amplification of high yields of uncontaminated mtDNA indicate that hair represents a useful and under-utilized source of aDNA. While the recovery of nuclear DNA from ancient hair is unlikely, this limitation also has the advantage of preventing the unintentional amplification of nuclear copies of mitochondiral sequences, which have proved problematic [8]. Furthermore, a preferential use of hair (and potentially feathers and scales) for genetic analyses would minimise the destruction of valuable historical and archaeological specimens caused by sampling of teeth or bones.

Supplemental data

Supplemental data containing experimental procedures are available at http://www.current-biology.com/cgi/content/full/14/12/R463/DC1/

Table 1: Details of ancient hair samples analysed.

Sample*	Species	Details†	Age‡	DNA§	Damage	Templatesß	Source
Tg415	*Homo sapiens*	Onge	50ßß	HVR1	0.0011	n/a	Lehrmann
Tg468	*H. sapiens*	Newton?	361-276**	No	n/a	n/a	Woolsthorpe Manor
Tg469	*H. sapiens*	Newton?	361-276**	HVR1	0.0007	7,200	Cullum Collection
Tg471	*H. sapiens*	Newton?	361-276**	No	n/a	n/a	Royal Society
Tg472	*H. sapiens*	Newton?	361-276**	HVR1	0.0019	5,700	Lord Portsmouth§§
Tg473	*H. sapiens*	Newton?	361-276**	HVR1	0.0015	116,100	Lord Portsmouth§§§
Tg474	*H. sapiens*	Newton?	361-276**	HVR1	0.0011	n/a	American Philosophical Society
Tg491	*Bos bison*	Dominion Creek	>64,500	CRS	0.0014††	75,600	Christie Mine, Dawson, YT
				16S	0.0050‡‡		
Pazyryk 1	*Equus caballus*	Ak-Alakha3	2800-2200	16S	0.0033	829,700	Molodin and Polos'mak
Pazyryk 4	*E. caballus*	Verkh Kaljin II	2800-2200	16S	0.0033	1,219,000	
Pazyryk 7	*E. caballus*	Ak-Alakha3	2800-2200	16S	0.003	2,141,500	
Pazyryk 8	*E. caballus*	Ak-Alakha3	2800-2200	16S	0.001	n/a	

*Sample: DNA extraction number. †Details: Original name of sample. ‡Age: Sample age in years. §DNA: Presence of amplifiable mitochondrial DNA in extract, either HVR1, 16S, or control region sequence (CRS). Where not indicated, HVR1 was not amplifiable. Damage: Damage measured as independent number of miscoding lesions per total bases of sequence amplified (excluding primer) [15]. If multiple extractions and amplifications have been performed, the average observed damage is given. ßTemplates: Approximate amount of amplifiable DNA fragments extracted from each 2 cm length of hair shaft analysed, as determined using Quantitative Real-Time PCR on serial dilutions of the 100 µl hair shaft DNA extracts (where analysed). ßß Years since sampled. **Age assuming hairs are authentically from Sir Isaac Newton. The bison hairs were sampled at Christie Mine, near Dawson, Yukon Territory, Canada. Bison CRS and 16S DNA sequences were independently replicated at two institutions, ABC Oxford and University of Copenhagen. The 16S mtDNA sequence matched that amplified from a bison bone previously extracted (BS200, M.T.P. Gilbert, unpublished data) and differed from *Bos taurus* at four sites. The control region sequence differed from *B. taurus*, and grouped phylogenetically within a large dataset of ancient bison sequences. Isotopic data are consistent with the identification of the sample as a bison ($\delta^{13}C$ = -21.9 ‰, $\delta^{15}N$ = 4.3 ‰), and is similar to modern and ancient herbivores [16]. §§ Hair attributed to Sir Isaac Newton as a youth. §§§ Hair attributed to Isaac Newton as an old man. For full details on all samples and sequences see Supplemental Data.

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