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# Comment on “Whole-Genome Shotgun Sequencing of Mitochondria from Ancient Hair Shafts”

Regis Debruyne,\* Carsten Schwarz, Hendrik Poinar

Gilbert *et al.* (Reports, 28 September 2007, p. 1927) reported that “hair shafts surpass comparably stored bone as an aDNA source [...] in regard to preservation and concentration of mtDNA.” When experimental parameters are carefully controlled for, including adequate sampling, quantitative polymerase chain reaction analysis, and modeling the decay of DNA, the general importance of this claim is not supported.

Bone has long been the preferred matrix for DNA extraction from ancient samples, due to its prevalence in archaeological and paleontological contexts as well as its presumed preservation potential (1, 2). It has also been used in metagenomic approaches to whole genome reconstruction of both Neandertals and mammoths (3–5). However, Gilbert *et al.* (6) have recently confirmed earlier work (7) showing that ancient hair shafts are good sources of DNA that can also adequately be used in 454 sequencing. The purported novelty of their recent study lies in the comparative analysis of mitochondrial DNA (mtDNA) sequence data from both hair shafts and bone. They analyzed relative mtDNA concentrations and “assessed the state of DNA preservation through [...] untrimmed read length and [...] cytosine-to-thymine (C→T) miscoding lesions” derived from 454 (8) sequence data alone.

We commend their use of hair for ancient DNA studies but question the rigor of their general statements concerning preservation (fig. S1). First, Gilbert *et al.* compared 10 hair shafts and a single bone, from separate individuals, thereby precluding any within-sample comparisons and allowing only cross-comparisons between different preservational histories. Second, the 454 library from bone derived from a nebulized DNA extract, a step omitted for the preparation of all hair shaft libraries. The nebulization shears DNA down to short fragments (400 to 800 base pairs), involves a substantial loss of DNA, and may relatively enrich libraries in high-molecular-weight contaminants (4). Although the effects of nebulization on damaged ancient DNA, as well as the generation of additional forms of damage, have yet to be explored, the rate of C>T damage (qualitative criterion used by Gilbert *et al.*) calculated for their bone library (1.699%) could not be reproduced when clones of a non-nebulized DNA

extract of the same bone from the same individual were sequenced. These yielded a rate of C>T as low as 0.483% (table S8), comparable to those from non-nebulized hair shaft samples [0.243 to 0.899%, (6)]. According to such a rate, the bone library no longer appears as an outlier in the thermal age comparisons (fig. S2).

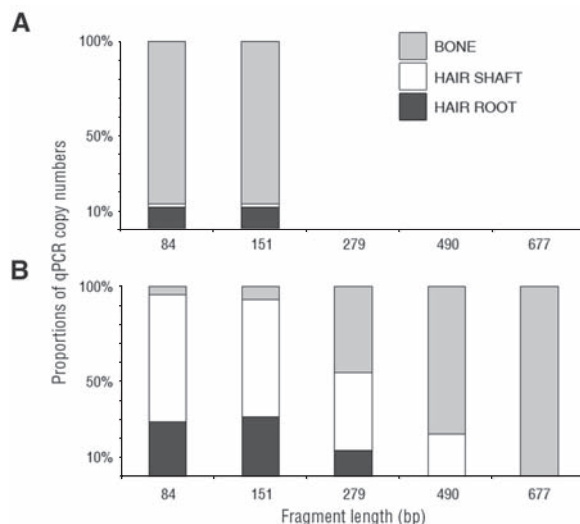
Third, hair shaft and bone libraries were sequenced on different genome sequencing (GS) systems. Whereas hair shaft libraries were sequenced using the Roche GS FLX [mean read length (MRL) expected: 240 base pairs (bp)], the bone library was sequenced using the Roche GS20 (MRL limited to 100 to 110 bp). MRL comparisons are thus artificial. Although Gilbert *et al.* (6) point out this limitation, they regard MRLs as their second qualitative criterion.

Last, the lack of experimental details in (6) precludes comparative analysis of some library specifics (supporting online text). To address these issues, we analyzed the mammoth mtDNA content of hair (shaft and root separately) and bone samples derived from the same individual: Lyakhov removed from the permafrost and preserved at room temperature since 1902 (9), as well as the Jarkov mammoth preserved frozen since its exca-

vation in 1999 (10). In addition, bone samples from Fishhook (11) and the 2005/915 mammoth (5) were extracted for comparison. DNA extracts were generated following published protocols (12) and used in a series of 5 mt elephant-specific quantitative polymerase chain reaction (qPCR) assays that span 84 to 677 bp of the *cytochrome b* (5). All qPCR results were scaled to copies per milligram of sample (12). Copy numbers from the varying fragment lengths were used to derive a degradation curve according to Deagle *et al.*'s model (13): a linear regression of log-transformed copy numbers onto amplicon sizes allowing the derivation of the amount of DNA molecules (N), the frequency of damage ( $\lambda$ ), and the mean amplifiable size ( $1/\lambda$ ).

Contrary to Gilbert *et al.*, our results (Fig. 1 and table S1) depict varying DNA concentrations in bone and hair, depending on the level of degradation. The mammoth DNA from Lyakhov is highly fragmented and damaged (positive PCRs for the two shortest products only) and almost entirely preserved within bone (86%, versus 12% in hair root and only 2% in hair shafts). In contrast, the DNA from the frozen Jarkov shows 10 to 20 times as many mtDNA copies in the hair shaft than in bone for the two shortest fragments, a result consistent with Gilbert *et al.*'s data. However, this trend quickly reverses with the 677 bp piece only amplifiable from bone (Fig. 1).

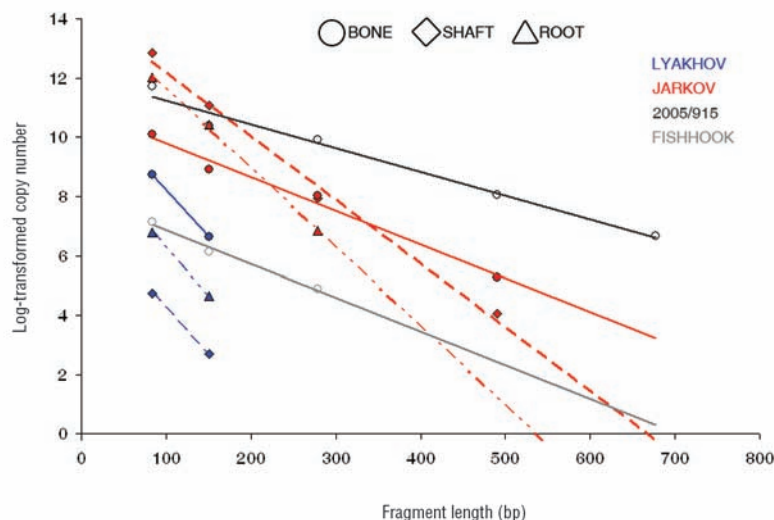
This quantitative trend in Jarkov illustrates a differential qualitative pattern of DNA degradation (Fig. 2). The steeper slope from hair shafts suggests more damage than in the bone ( $\lambda$ ), as does the mean amplifiable fragment length (twice as long in bone) (table S1). It shows that short amplifiable fragments are the norm in hair shafts, consistent with Gilbert *et al.*'s short MRLs (range: 60.5 to 129.8 bp, 6 out of 10 actually being worse than the nebulized bone sample at 101.1 bp). On the other hand, all DNA extracts derived from frozen bones show similar patterns of DNA damage with consistently lower  $\lambda$  values, although their endogenous DNA concentrations



**Fig. 1.** Quantitative analysis of ancient mammoth DNA extracted from hair and bone from the (A) Lyakhov and (B) Jarkov specimens. Proportions of qPCR copy numbers for all mitochondrial assays shown per fragment size (given in bp) and tissue type extracted.

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**Fig. 2.** Qualitative analysis of ancient mammoth DNA extracted from bone and hair presented as mitochondrial DNA degradation profiles as estimated per Deagle *et al.*'s regression model (13). Regression lines are shown for all extracts (see table S1 for original data).

[N, (13)], vary widely (Fig. 2 and table S1). As the current development of high-throughput sequencing technologies is targeting ever-longer reads, the apparent preservation advantage of bone over hair shafts might become increasingly relevant in the future.

Paired tissue type comparisons thus confirm that ancient hair shafts may indeed contain large amounts of mtDNA, but they also reveal that the quantitative and qualitative superiority of hair shafts over bone does not appear to be a general

feature. Our analysis supports that the chances of retrieval of endogenous DNA are not higher in hair than in bone.

The field of ancient DNA has always been plagued by controversial conclusions leading some authors to establish a set of experimental criteria (2). Although those criteria need to be revisited with the advent of paleogenomic studies, proper experimental controls remain a necessity to ensure the applicability of generalized comments about DNA preservation.

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Materials and Methods

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References

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