Response to Comment on "Whole-Genome Shotgun Sequencing of Mitochondria from Ancient Hair Shafts"

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Response to Comment on "Whole-Genome Shotgun Sequencing of Mitochondria from Ancient Hair Shafts"

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Debruyne *et al*. challenge the findings of our study and imply that we argue that hair shafts are an overall superior source of ancient DNA than bone. However, the authors are misreading and misinterpreting the conclusions of our study; we claim nothing further than that hair shaft represents an excellent source material for the shotgun sequencing of mitochondrial DNA genomes.

Debruyne *et al*. (*1*) raise a number of points that, if accurate, certainly support their viewpoint that bone has many advantages over hair. In particular, they demonstrate experimentally that endogenous DNA sequence reads of bone are in general longer than contemporary sequences from hair shaft. Furthermore, they demonstrate that per unit mass of tissue extracted, DNA concentration is richer in bone. With regard to both these points, we are in complete agreement. Indeed, a third benefit of bone over hair in the context of our study is its vast dominance in the fossil record; there is simply more of it available, which naturally enables a larger number of studies to be undertaken. Thus bone, has several advantages over hair shaft as a source material.

It is perhaps not surprising, therefore, that we do not in our previous article claim anything to the contrary with regard to these points. Debruyne *et al*.'s (*1*) analyses show how bone outperforms hair with regard to higher yields and longer fragments of DNA. In our report, we made no claims on DNA concentration from hair in comparison to bone, nor did we make any claims on superior fragment lengths in hair in contrast to bone. Furthermore, we did not use it as a quality index. On the contrary, we stated that "We observed an average sample-dependent mitochondrial read length between 60.5 and 128.1 bp. The previously described average read length of 101 bp from a bone sample was limited by the instrument read length (Roche GS20), leaving open the possibility that the bone sample retained longer fragments of mtDNA than those that we observed." Indeed, we would be surprised if sequence lengths were longer in hair shaft extracts, because a number of studies have argued that endogenous DNA undergoes damage during the keratinization process [e.g., (*2*)].

In light of the clear discrepancy between our published statements and the arguments raised by Debruyne *et al*. (*1*), it is worth reclarifying our previous claims: simply that hair shaft offers an excellent source material from which to generate complete mitochondrial DNA (mtDNA) genomes using the shotgun sequencing method offered by the Roche GS FLX platform. There are several reasons that hair shaft is an excellent source, and these may be interpreted as being "better" than for bone, in the context of the application. Specifically we refer to the following:

First, the endogenous mtDNA content of hair shaft, as observed among Roche GS FLX sequences, is much higher that from bone: Up to 2% of the sequences generated by the Roche GS FLX sequencing are mtDNA (*3*–*5*), in contrast to 0.08% in the extremely well-preserved deep-frozen bone sample chosen by Poinar *et al*. (*6*) for the first paleogenomic study. Although this may differ to the mtDNA level in raw extracts, our study (*3*) was concerned with final outcome. Thus, in the context of shotgun sequencing, the purpose of our study, this enrichment is a clear advantage.

Second, our comparison of the levels of postmortem damage–derived Type 2 (C→T, G→A) miscoding lesions between hair and bone demonstrates that levels are higher in bone material. Although we did not previously perform this comparison on paired samples (due to sampling limits), our analysis factored in the thermal age of the specimens examined, a key point that Debruyne *et al*. (*1*) neglect. Temperature plays a key role in the rate of such deamination reactions (*7*), and our data clearly indicate that for any given temperature, the levels of observed damage are lower. It is interesting that Debruyne *et al*.'s comparison from paired samples appears to show that damage rates do not differ. The authors suggest that nebulization during Roche GS FLX library preparation may explain our previous observations. This seems unlikely. Indeed, a paper coauthored by Poinar specifically investigated, and rejected, this hypothesis (*8*). We instead question the method that Debruyne *et al*. (*1*) used to generate their data; it is an accepted fact that conventional polymerase chain reaction (PCR) and cloning is a significantly more biased technique than processes involved in Roche GS FLX library generation.

Debruyne *et al*. (*1*) raise several other points in their article that we can also briefly address. It is true that we did not provide specific information as to quantities of hair shaft used and the total reads per library. This information reflects the total amount of DNA that can be extracted from a sample, the size of the emulsion PCR library, and the skill of the manipulator. This has little to do with the focus and claims of our study, which deal with relative (as opposed to absolute) comparisons. Furthermore, we did not discuss the nuclear DNA (nuDNA) content, due to its lack of relevance in the context of mtDNA genomes. We are pleased to reveal, however, that the nuDNA content of the samples is extremely high, consistent with observations of the ease with which hair shaft can be decontaminated from exogenous DNA sources (*9*–*11*).

In conclusion, we stand by our original claims and subsequent demonstrations (*4*, *5*) that hair shaft represents a unique source for paleogenomic studies. Where present, it can be sampled easily, causing minimal visual damage to specimens, and subsequently used as an efficient means to recover pure endogenous DNA, which in turn can be used by the Roche GS FLX platform to generate complete mtDNA (*3*–*5*), or even nuDNA genomic information. Ultimately, however, the debate about which set of conditions favor bone or favor hair will only be settled by a systematic study that generates and analyzes a large amount of DNA sequence from several tissues and a variety of specimens.

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