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Response to Comment by Poinar *et al.* on "DNA from Pre-Clovis Human Coprolites in Oregon, North America"

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The arguments of Poinar *et al.* neither challenge our conclusions nor would contribute to the verification of our data. We counter their questions about the authenticity of our ancient DNA results and the reliability of the radiocarbon data and stand by the conclusion that our data provide strong evidence of pre-Clovis Native Americans.

In our study of the Paisley Cave coprolites (*1*), we applied some of the most comprehensive controls yet applied to ancient DNA. These controls were stricter than those used by our critics in related studies on coprolites, ancient humans, or both (e.g., *2*–*5*). Nevertheless, Poinar *et al.* (*6*) challenge our study on several grounds. Although ancient human DNA results can rarely be 100% certain, and studies presenting noteworthy conclusions should be challenged, the arguments presented in (*6*) do not undermine our claims.

The initial arguments by Poinar *et al.* (*6*) focus on sample contamination. Contamination has two contexts with regard to our study. The first is that most commonly raised in ancient DNA (aDNA) studies—the recent contamination of ancient human samples. Given the absence of evidence of laboratory-derived contamination, we acknowledged (*1*) that two likely sources exist: unprotected handling at excavation or leaching while still in the ground. As we stated, the samples were contaminated through handling, although not by sources of haplogroup (Hg) A and B mitochondrial DNA (mtDNA). Poinar *et al.* (*6*) question why we did not resolve who the contaminators were. As detailed in (*1*), the levels of contamination and DNA degradation made this impossible. Before we address the issue of leaching, we highlight that there is an additional form of contamination of equal importance: contamination at time of origin. In a cave inhabited contemporaneously by multiple individuals (possibly with canid companions), speculation based on human behavior would render it surprising if the inhabitants did not defecate/urinate in collective localities. We stated this in our original text, presenting it as a reason for the presence of canid DNA in some of the samples. We also note that this scenario would still require a human presence and thus is consistent with pre-Clovis human occupation. As such, the controls in our study were principally focused on the former (countering leaching and modern handling). Poinar *et al.*'s statement that a central tenet of our hypothesis requires the presence of only one Native American sequence in each sample (*6*) is inaccurate. The central tenet of our hypothesis is that combined genetic, nongenetic, and other evidence indicates that recent contamination with Hg A or B sequences is unlikely.

Poinar *et al.* further note that we neglected to provide primer sensitivity/optimization data or to use quantitative polymerase chain reaction (qPCR). Ignoring the fact that such data are rarely provided in genetic studies, including (*2*–*4*), this challenge is not problematic. Our initial assay, multiplex PCR with minisequencing (MPMS), is a sensitive tool that detects contaminants at a 4% threshold in aDNA extracts (*7*). Although our PCR targets ranged from 50 to 105 base pairs (bp), copy number of aDNA molecules increases exponentially as size is decreased (*4*, *8*). Thus, one might expect the smallest amplicons to be most sensitive. In addition, Poinar *et al.* fail to observe that many of our results, in particular both the MPMS and cloned Hg B results, derive from our longest amplicon. Size notwithstanding, the relative sensitivity of the different primers themselves is also irrelevant here, because the MPMS assay requires all eight primers to coamplify; the output is always eight sequenced single-nucleotide polymorphisms (SNPs), which simply differ by the state of each SNP. The only conceivable way that sensitivity could be a problem in this context would be if the particular derived versus ancestral SNP allele could sufficiently affect the binding of primers that are positioned multiple bases away, which is highly unlikely. With regard to a lack of qPCR, we are unclear what the grounds for this argument are. Historically, qPCR has been used to prevent sequence errors derived from postmortem miscoding lesions. However, we adopted a simpler, and widely accepted, alternative—the reproducibility of data. Indeed, Poinar and colleagues have used this in several of their own studies on coprolite aDNA (*2*, *4*).

Perhaps the biggest challenge facing our study was proving that modern DNA had not leached into the coprolites. Although the only comprehensive means to conclusively rule out leaching is screening all the cave soil for contaminants, this is not realistic. We agree that, on their own, some of the arguments against leaching are not watertight (e.g., previous observations on DNA movement in temperate soils and the diagnostic power of protein). However, in combination, we believe our arguments suggest that leaching is unlikely. We do not feel, furthermore, that the criticisms offer any additional solution to the problem, and we disagree with several. It is not unusual that only 13 of 28 soil controls tested positive for human DNA, given that, in contrast to the coprolites, they were not directly handled but simply sampled into storage containers. With regard to the wood rat primer sensitivity, as detailed in (*1*), they are effective on ancient wood rat coprolites from the site, and thus clearly work. Given this, and that such coprolites constitute up to 80% of the sediments in the cave, we argue that leaching is unlikely.

Poinar *et al.* (*6*) also raise questions about the Paisley Caves site, assemblages, and ^{14}C dating. Direct dating indicates that the pre-Clovis assemblage includes a stemmed point, five nondiagnostic chipped stone tools, debitage, a hand stone with horse protein residues, and a butcher-cut grouse sternum. The assemblage is not Clovis but is Paleoindian (*9*). Obsidian pre-Clovis artifacts were subjected to hydration dating (OH). OH rate variability is caused by a combination of inherent characteristics and environmental variables (*10*). Controlling for effective hydration temperature (EHT) and employing mean group OH measurements rather than individual measurements often greatly improves OH rate accuracy and concordance between ^{14}C and OH results (*11*–*14*). EHT has been calculated for multiple microsettings at the site by recording temperatures every 45 min between 2005 and 2008. Although more work needs to be done, we have observed good concordance between matched OH and ^{14}C dates. With regard to sedimentary disturbances, these were generally traceable in Cave 5 (*13*). Sediments dip and thicken differentially toward the cave center, causing substantial elevational variation among penecontemporaneous specimens. Site formation processes caused occasional intrastratigraphic age reversals. However, the general

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integrity of deposits is well supported by the majority of stratigraphically correct dates obtained on artifacts, bone collagen, and human coprolite dates.

Our dated samples from Paisley Caves consisted only of identifiable fibrous plant matter carefully extracted from the human coprolites, as stated in (*1*) [see the materials and methods and figure S2 in the supporting online material for (*1*)]. In their previous published work [e.g., (*4*)], on the other hand, Poinar and colleagues homogenized coprolite remains before accelerator mass spectrometry (AMS) dating. We suspect this to be the source of their misinterpretation and confusion over our results. The $\delta^{13}C$ values we published do not relate to bulk coprolitic carbon, and it is, therefore, impossible to infer general characteristics of human diet from them and to then link this with possible lacustrine reservoir effects. The C3 plant $\delta^{13}C$ values do not provide evidence that the humans are herbivores, as Poinar *et al*. (*6*) imply. We selected only plant matter from the coprolites for dating to avoid potentially problematic bulked samples, which could conceivably include carbon from soil or sediment of different age. The variation in the two $\delta^{13}C$ values for specimen 1374-PC-5/5D-31-2 may well reflect small amounts from two different types of plant matter being extracted and included in the analysis, for example C3 ($\delta^{13}C$ ranging from ~−20 to −35 per mil) and C4 plants (~−11 to −15 per mil). Further material from 1294-PC-5/6B-40 is currently being AMS dated to confirm which of the two ages thus far obtained (Beta-2134231 and OxA-16376) is more reliable. We consider a freshwater reservoir effect to be unlikely in explaining this difference, or to have a major effect on the other results, but we will report new dating results for this specimen in due course. We contend that the radiocarbon results are (i) reproducible between two independent laboratories (with the exception of the 1294-PC-5/6B-40 sample noted previously); (ii) not subject to a reservoir effect because they yield $\delta^{13}C$ values, which are predominantly indicative of terrestrial C3 plant remains; and (iii) of fibrous plant material which ought to give reliable AMS determinations when effectively pretreated.

In summary, although we accept that the sample contamination makes our data set far from perfect, we feel that the arguments presented in (*6*) would neither help resolve the data nor seriously challenge our conclusions. Ultimately, perhaps the only resolution may come from new, sterile excavations at this unique site.

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