DNA PROFILING OF THE BABES IN THE WOODS

Verbatim transcript of Dr. David Sweet's[1] explanation of the DNA extraction process in the "Babes in the Woods" case (Interview, Vancouver Police Centennial Museum, 2004):

In a lot of cases we were dealing with at the time [when BOLD received the Babes in the Woods material in 1998], we were finding it very difficult to extract DNA from skeletal remains. When you're only dealing with bones or with teeth, we didn't have any techniques that were available yet that would really allow us to get a good sample. So my lab was one of the very few labs that were working on that problem. We deal with bones cases all the time. And so it was us that were really faced with the problem of how to get better results. And I am very proud to say that it was in my lab as a result of work with my DNA technician that we were able to perfect the technique through a process called "cryogenic grinding" - freezing the sample to a very low temperature so that it gets very brittle and then breaking it into very tiny particles so that you can expose the DNA and extract it and analyze it.

So we had done some cases like it. It was working very well. And Sergeant Honeybourn decided that he wanted these samples at the lab in order to see what could be done with them. So we were in a situation where we were going to apply this brand new method to these very old samples.

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[1] **Dr. David Sweet, world-renowned forensic odontologist.**
"Dr. Sweet was the driving force behind the Bureau of Legal Dentistry lab at UBC [The University of British Columbia]. BOLD is Canada's first facility devoted exclusively to police work, research and instruction in the use of forensic dentistry for crime investigation and prosecution… BOLD also educates and creates awareness among dentists around the world by offering courses in forensic odontology, courses that Dr. Sweet designed and delivers."
Vigna, J. (2005, Summer) The Sweet Spot, Trek- the Magazine of the University of British Columbia, *60*(2), 22-25

And I want to tell you that it was a real challenge because I didn't know if we were going to get a result from such old materials. And I was the first one that was very excited about the fact we were able to process them and produce the result.

Now the technique involves trying to decide which is going to give you the best potential result and we know there is a lot of DNA in teeth, so I focused on the teeth in this case. And the reason for that is that there were baby teeth present and that is an area of the body that is very rapidly growing and very rapidly developing in the living child. So I thought if there was going to be a good source of DNA that would be a site that we should focus on. And it turned out that I was correct.

So we took out some of the baby teeth and processed them through this new technique called "cryogenic grinding." We pulverized them into a very fine powder and then extracted enough DNA to run the sample.[2] *And we produced a full DNA profile*[3]*, which also includes the gender of the individual. We were able to tell male from female*

[2] "DNA has been previously recovered from human teeth for RFLP and PCR-based forensic analysis. In some cases, the maximum amount of undisturbed tooth structure is required for ulterior forensic analysis. But, in most cases, following comprehensive documentation, it is possible to section the tooth longitudinally or horizontally, or crush it to access the DNA-rich core. This [is] an alternative method to recovering DNA from whole extracted human molar teeth. A 6700 freezer mill was used to pulverize 20 teeth under frozen preparation in liquid nitrogen and sterile conditions. The mean yield of DNA was 30.9 mg (18.4 mg DNA per gm tooth powder). The resulting fine powder [is] subjected to organic extraction and subsequently quantified using slot blot hybridization… The technique is simple and relatively rapid. Isolation of the samples during pulverization minimizes the risk of contamination."
Sweet, D., Hildebrand, D. (1998) Recovery of DNA from human teeth by cryogenic grinding (abstract), Journal of Forensic Sciences, *43* (6), retrieved September 12, 2006 from http://journalsip.astm.org/JOURNALS/FORENSIC/PAGES/3064.htm

[3] "**Anatomy of a profile.** Individual features such as eyes, nose, ears, hair, and accoutrements help us to differentiate one person from the next. With enough features we can be confident in the identity of any particular person. Genetic markers work in exactly the same way. With enough loci (assuming no laboratory error and disregarding twins), we can become convinced that a sample originates from a particular source."
From Rudin, R., Inman, K. (2002) An Introduction to Forensic Analysis. Boca Raton, Florida: CRC Press LLC

almost immediately. And in fact, one of the conclusions that we drew was that initial tests that had been run were false- the conclusions that were developed were not accurate- that one child was a female and one child was a male and that they may be related genetically in the form of fraternal twins.

It turned out from our genetic analysis that in fact both the remains were from boys and they were not twins- that were in fact brothers with a single common parent. And so the conclusion was that they were born to the same mother but had different fathers. So we were very excited about that and I phoned Sergeant Honeybourn in order to let him know the results. I asked him right at the time I was able to get him on the phone if he was sitting because, after all these years of investigation, I thought that the news that one child was not female but in fact male would change the scope of the investigation. And it did. Although we look back at that now and think- why didn't we start to look for two missing boys as well as a boy and a girl- that's easy to say. There was such good evidence at the time that the whole investigation was focused in that direction.

What I mean by a full [DNA] profile is that we have results from all the different genetic sites that we tested. From a scientific point of view, this is very good because if there was an opportunity to compare these DNA samples to potential parents or others to identify the children, we have a lot of different genetic information that we can use and the statistical calculations that can be done to confirm our results are very valid when we've

got lots of this data. But there are some cases in forensic science where you only get what are called "partial profiles" where, because the DNA is so degraded and because it is such an old sample that you actually don't get genetic information of all the different sites. The tests that we ran looked at ten different sites on the chromosomes and we have results from all ten of those. One of them is gender. So we now have nine additional genetic sites that we can compare to potential others to identify the children. A partial profile may involve only two or three, or may be even six or seven, of these points, but we're very happy to have all nine, plus gender.

[We determine the ages of the children] by their dental development. The degree of development of the permanent teeth that were still embedded in the jaw bone underneath is known to occur at a certain pace and so by taking X-rays of the jaws and looking at that and analyzing the amount of development of the adult teeth, we were able to come up with a pretty accurate estimation of what that age would be. Now sometimes the chronological age- the age since the actual birth- is different than your dental age or your anatomical age because there are different factors that can affect people that don't age as quickly, or age more quickly, because of environmental effects. But that's not usually true in young children.

Usually as an older adult, you might get inaccuracies in your dental estimates and trying to compare that to the chronological age. But with kids this young- I think one was age six and one was age seven approximately,[4] *we can be*

[4] Dr. Sweet's final report indicated that the older child ("Skeleton 1") was 7.3-8.7 years and the younger child ("Skeleton 2") was 7.0 to 7.5 years.

pretty accurate with a young child, plus or minus six months, because there are tables that actually are published about dental development that are fairly accurate.[5] *Sometimes when we're estimating in a mid adult, say thirty five to fifty years of age, we're sometimes estimating plus or minus five to ten years. We can't be that accurate. But with these younger children we can certainly be quite a bit more accurate.*

In terms of comparing and trying to identify the children, the best chances that we have for that now, because we're using what is called a "nuclear DNA profile", is the immediate family- the mother, the father, sisters or brothers. The immediate family would be the best source. But, because we have DNA, we also have the opportunity to sequence genes that are in the chromosomes and do what is called "mitochondrial DNA analysis" and in that case we can have any maternally related aunt or grandmother, or even a male relative but that's from the mother's side of the family. [6] *And because the mitochondrial DNA is always passed down from the mother, and never from the father, we can go back in that lineage much further than the immediate family. Now we haven't exactly done that sequencing yet, but because we've had the DNA available now, we can do that with modern technology.*

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[5] "Between ages 6 and 7 years of age there are: 20 deciduous teeth, 4 first permanent molars, 28 permanent tooth buds in various states of development." CJ (1998) Occlusion and Dental Development Retrieved September 15, 2006 from http://www.uic.edu/classes/orla/orla312/OCCREVIS.htm

[6] "The vast majority of the genetic material in the human genome resides in the nucleus of each cell… the **nuclear DNA.** Some additional bits of genetic material exist, however, and are contained in other subcellular compartments called **cell organelles.** One of these is the **mitochondrion** (pl. **mitochondria**), in which some of the processes of cellular respiration take place… Nuclear DNA is inherited from both parents… Due to the technicalities of fertilization, genetic material from mitochondria [**mtDNA**] is inherited only from the egg cell of the mother; thus mtDNA is said to exhibit maternal inheritance."
From Rudin, R., Inman, K. (2002) An Introduction to Forensic Analysis. Boca Raton, Florida: CRC Press LLC