Extracting Ancient Dental DNA: Just Like Pulling Teeth

ANNE STONE, ARIZONA STATE UNIVERSITY

HOW HARD CAN IT BE to extract DNA from the teeth of chimpanzees that died only 30 or 40 years ago? These were my initial thoughts when presented with the opportunity to examine the DNA of the most famous chimpanzees in the world. These individuals, studied by Dr. Jane Goodall at Gombe, included Flo, Madam Bee, Satan, Hugo, and Getty.

At the time of her initial pioneering work, collecting genetic data was not part of the standard primatology repertoire. Today, all of the chimpanzees at Gombe are genotyped for autosomal microsatellites to see how they are related to each other (and, in particular, to figure out paternity). Such analyses began there in 1991 with the development of non-invasive methods of DNA sampling such as using shed hair collected from night nests or using fecal samples. These data have been key for testing hypotheses about kin selection, social structure, mating patterns, and reproductive success.

Assessing the same microsatellites in some of the early individuals would let us add to the existing pedigree. In addition, we could see whether inbreeding is increasing (because of increasing isolation from other chimpanzee communities) by comparing the genetic diversity within the community over time. Finally, maybe we would be able to retrieve SIV (simian immunodeficiency virus, found there today) to see who was infected and how the virus has changed over time...if the preservation were sufficient.

Alas, it was not. Ancient DNA work is some of the most frustrating research there is (though perhaps some of the paleoanthropologists will disagree as they search long and hard for fossils!) This frustration I learned twenty years ago when I was in graduate school working on the genetic analysis of a prehistoric Native American population, with funding from The Leakey Foundation, to see how members of that community were related to each other and what their genetic diversity told us about the peopling of the Americas.

Ancient DNA analyses are challenging for several reasons. First, the DNA is degraded, usually into pieces that are smaller than 100 bases long. Second, it is damaged; this can cause the enzymes used to copy the DNA to make mistakes or not work at all (for example if the DNA fragments are tangled into chemical knots that the enzymes can't attach to). Finally, contamination is one of the greatest concerns, so researchers in the field take great precautions to prevent modern DNA from entering their ancient DNA labs.

[continued on page 3]
A Letter from the President...

SPAIN AND GIBRALTAR offer breathtaking scenery and wonderful food and wine, but what made the Fellows Tour of Spain and Gibraltar (May 18–25) so extraordinary was the opportunity to connect with scientists researching the last million years of human prehistory and to hear about their discoveries long before the news hits the New York Times.

One such opportunity came when research scientists personally showed us the originals of the oldest cave art in Europe, possibly the world. These crimson disks and hand stencils in El Castillo Cave are dated to at least 40,800 years ago, making them at least 4,000 years older than the next oldest artwork from Chauvet Cave in France. This is the period when the first modern humans were moving into Spanish areas already occupied by Neanderthals, and this art is very different from later art emphasizing animal depictions. Were these figures the earliest art by modern humans or the last art of the Neanderthals?

Another exciting opportunity was our visit with Professor Manuel González Morales, co-director of El Mirón Cave, and long-time colleague as well as Leakey Foundation grantee Lawrence Straus. Last autumn the team continued excavation on the remaining deposits where a Magdalenian woman was reburied by her tribe, after her bones and jaw had been collected and then stained with red ochre. The El Mirón team is working with Svante Pääbo from the Max Planck Institute to extract nuclear DNA from her remains. Along with already collected and analyzed mitochondrial DNA from the “Red Lady of El Mirón,” what exciting new information will these data offer?

Equally intriguing was the research being conducted in Gibraltar, by Clive and Geraldine Finlayson, on Neanderthals who occupied caves by the sea and lived a life of maritime hunting and fishing, which is more commonly associated with modern humans. Have we given the Neanderthals the credit they deserve? Since it is now known that many modern humans continue to carry a small percentage of Neanderthal DNA, perhaps it’s a good time to re-evaluate our perception of these often disparaged individuals.

The Leakey Foundation has made a commitment to investing in field work like the projects at El Castillo, El Mirón and Gibraltar, allowing the world to better understand our earliest human story.

Another location where science has benefited from almost a million dollars in Leakey Foundation funds for field research is South Africa. I am pleased to announce that South Africa is the destination of the 2015 Fellows Tour. The journey will take place May 3–13 and will take travelers from Cape Town to Johannesburg to see the most extraordinary rock art, archaeological, and paleoanthropological sites, while enjoying world famous wines from Stellenbosch and the vibrant cultural history of the country. As is the case with all Foundation tours, we will meet the researchers making the discoveries.

I hope you’ll join us!

Donald E. Dana
President of The Leakey Foundation

Registration for the Fellows Tour of South Africa opens at the end of September. For trip updates visit: leakeyfoundation.org/travel
However, over the last twenty years, the changes in molecular biology and in the field of ancient DNA have been dramatic. When I began my dissertation, PCR (the polymerase chain reaction) was cutting edge technology, and it allowed us to amplify small fragments of DNA from specific regions of the genome (which was not yet completely sequenced)! It often took weeks or months to amplify and sequence the five small fragments that I targeted in order to piece together a ~350 base pair sequence of the mitochondrial hypervariable region, and I often ran out of DNA before I was successful. Today my students and I can extract DNA and turn it into a DNA library. In essence, this immortalizes the DNA so that we can go back to it repeatedly for different experiments. We can also use DNA baits to “fish” out only the DNA fragments in the library that we are interested in, and then we can sequence those fragments of DNA using high throughput sequencing technology. Thus, we can target the entire mitochondrial genome (~16,500 bases long) and sequence it (along with many other samples) in only one sequencing run!

So why hasn’t this new technology worked (yet) for the Gombe samples? Ancient DNA is best preserved in cold, dry places with a neutral or slightly alkaline pH and minimal temperature fluctuations. Gombe is not one of those places. These individuals were buried in the ground for approximately 1-3 years, and then their skeletons were curated for further study. Dr. Mike Wilson (University of Minnesota) worked with us to select and scan the tooth samples, and then my laboratory extracted the DNA from tooth roots, which typically preserve DNA best because they are so hard. Our results to date indicate that these samples have very little DNA. However, we have not yet lost hope. Recent methods developed to recover smaller fragments of DNA (30-70 base pairs in length) were employed successfully with samples from Sima de los Huesos (which are over 300,000 years old.) These results offer the possibility that we will still be able to obtain DNA from the Gombe samples. We are currently testing those methods and we will keep you posted as to our success!

Snapshots from the Fellows Tour of Spain and Gibraltar

Martha Lewis on her way to Covalanas.

Cole Thomson, Pat Poe, Maria Martinon-Torres, Don Dena, Sage Johns at Atapuerca.

Neolithic hand stencil, El Castillo. Photo: Roberto Ontañon Paredo

Ken and Janice Kaye celebrate their anniversary.

The Fellows Tour group at the Menga Dolmen.
**Awarded Grants**

**Spring 2014**

**Behavioral**

**Andrew Cunningham**, Harvard University  
*Food-plant productivity for female foragers: Function of water regime*

**Horacio de la Iglesia**, University of Washington  
*Searching for ancestral sleep in human populations of the Argentinean Chaco*

**Katerina Fliegerova**, Czech Academy of Sciences  
*Symbiotic anaerobic fungi in the digestive tract of hominines*

**Stanislaus Kivai**, Rutgers University  
*Fallback foods implications in juvenile Tana River mangabeys, Cercocebus galeritus*

**Elizabeth Renner**, The George Washington University  
*Individual, observational, and imitation learning in humans and great apes*

**Aaron Sandel**, University of Michigan  
*The development of social bonds in male chimpanzees*

**Stacey Tecot**, University of Arizona  
*Evolution of allomaternal care in red-bellied lemurs, Eulemur rubriventer in Madagascar*

**Paleoanthropology**

**Justin Adams**, Monash University  
*The Haasgat hominin site (South Africa): excavation and palaeobiological integration*

**Sergio Almécija**, Stony Brook University  
*Miocene apes from Castell de Barberà (Catalonia, Spain)*

**Benjamin Collins**, University of Toronto  
*Middle Stone Age excavations at Grassridge Rockshelter, SA*

**Avi Gopher**, Israel Prehistoric Society  
*Excavating Middle Pleistocene Yabrudian and Amudian at Qesem Cave, Israel*

**Daniel Green**, Harvard University  
*Plio-Pleistocene seasonal precipitation through isotopes in bovid molars, West Turkana*

**Nicholas Holowka**, Stony Brook University  
*Kinematics of the chimpanzee foot during terrestrial and arboreal locomotion*

**Julien Louys**, The Australian National University  
*Palaeontological and archaeological investigations of Pleistocene cave deposits from Sumatra*

**Jason Massey**, University of Minnesota  
*Pattern and timing of ontogeny in populations of Gorilla & Pan*

**Isaiah Nengo**, De Anza College  
*Preliminary palaeontological exploration at Napedet: A new middle Miocene site in the Turkana basin*

**Allison Nesbitt**, Stony Brook University  
*Morphological integration between the face and dentition throughout ontogeny*

**Dillon Niederhut**, University of California, Berkeley  
*MRI microscopy of human motor neurons*

**Oliver Paine**, University of Colorado  
*Investigating the nutritional/mechanical properties of south African savanna plants*

**Ryan Raam**, City University of New York  
*Seeking more descendants of the pre-agricultural populations of East Africa*

**Kenneth Rose**, Johns Hopkins University  
*Basal euprimates from the early Eocene of Gujarat, India*

**Mohamed Sahnouni**, CENIEH  
*Homo erectus behavior and adaptation: Reinvestigating the hominin site of Tighennif (formerly Termifene), Algeria*

**Gonen Sharon**, Tel Hai College  
*Excavating the Mousterian hunting camp of NMO, Upper Jordan River*

**Nathan Thompson**, Stony Brook University  
*Kinematics and evolution of upper body stability in hominins*

**Alexandra Uhl**, Eberhard Karls Universität Tübingen  
*Sex determination in geographically and ontogenetically diverse samples*

**Peter Ungar**, The University of Arkansas  
*Hadza dental microwear: Implications for the evolution of human diet*

**Bence Viola**, Max-Planck-Institute of Evolutionary Anthropology  
*Middle Pleistocene hominins in Central Asia - Excavations at Sel'ungur*

**Ian Wallace**, Stony Brook University  
*‘Phylogenetic signal’ in limb bone shaft structure among South Africans*
Baldwin Fellows

Dagmawit Abebe
City University of New York

Simone Dagui Ban
Max-Planck-Institute of Evolutionary Anthropology

May Murungi
University of the Witwatersrand

Justin Pargeter
Stony Brook University

Moreen Uwimbabazi
Harvard Graduate School of Arts and Sciences

Aaron Sandel observing a chimpanzee in the field. Photo: Aaron Sandel

To read the final reports of grants we have funded visit leakeyfoundation.org/reports

Stacey Tecot, in the field. Photo: U. of Arizona

Excavating the Mousterian hunting camp, Upper Jordan River. Photo: Gonen Sharon
THE LEAKEY FOUNDATION'S Annual Fellows Dinner and Auction was held on Friday, April 25, 2014, at the historic Carolands Chateau in Hillsborough. The event is a way to acknowledge the Foundation's patrons whose generous support allows us to continue the mission of our organization.

The Carolands Chateau is a masterpiece of American Renaissance and Beaux Art architecture, built by the heiress of the Pullman car fortune, Harriet Pullman Carolan. The original estate sat on 500 acres, with the home boasting 67,000 square feet and 98 rooms. In 1998, after years of neglect, Charles and Ann Johnson purchased the estate and remaining land and, together with famed designer Mario Buatta, began an extensive restoration. Recently, the Johnson's established the Carolands Foundation to keep the property preserved in perpetuity, and occasionally they allow charities to host events at the estate. The Leakey Foundation is honored to have hosted our special event in such a beautiful and historic place.

This year marked the 44th anniversary of the Fellows Dinner; the gathering of 80 guests included scientists and the donors who provide indispensable financial resources making possible vital research and exciting new outreach programs. Three special guests, Mary Alice and William Yund along with Jim Carthy, were acknowledged for their ongoing dedication, having donated to the Foundation annually for over 25 years.

A new component of the dinner was the addition of a raffle for two wine collections. Members from the Foundation's Scientific Executive Committee, Dr. Joan Silk and Dr. Alexander Harcourt, selected the winning raffle tickets. Jeanne Dana won the Cade Estate Winery collection, and Sally Gallagher won the PlumpJack Winery collection.

This year's auction lots included (among others) a case of some of the top wines from Northern California; a cast of the famed Taung Child skull made from the master positive; and stays at beautiful locations including San Miguel de Allende, Mexico; a ranch in Cody, Wyoming; a historic Queen Anne home on an estate along the Russian River in Healdsburg; a five-star resort in Napa; and a scenic farmhouse in Provence.

Another unique auction item was the chance to name a wild baby chimp at the Ngogo research site in Uganda. Nina Carroll won this rare opportunity and allowed her granddaughter, Daisy, to select the baby boy's name, and she selected "Jumbo." Daisy will receive reports on Jumbo's life at Ngogo and photos of Jumbo with his extended family, including his mother, two brothers, a sister and her two children.
For many though, the bidding turned most competitive when a homemade lemon meringue pie (made by Life Trustee George Smith) was offered for auction. Jeanne Newman was the lucky winner, and the pie was served to her table during dessert...her table mates couldn’t have been happier with a slice of her winnings!

Our sincere thanks to the generous donors who contributed items for the auction, and to those bidders who helped us reach our fundraising goal of $20,000. (The auction and raffle proceeds bear no administrative burden, so 100% of these funds will be used to award more research grants.)

We are grateful to Aaron Bastian of Bonhams for participating, once again, as our auctioneer. And a very special thanks to Meg Starr and her incredible team for making this event a true success!

To learn more about becoming a Leakey Fellow, please contact Sharal Camisa or visit leakeyfoundation.org/donate

All photos by Moanalani Jeffrey.
The dialogue was engaging and provided many of the participants with creative ideas and the hope of collaborating together in the future. While there were many tangible outcomes, here are a few highlights from our discussions on how best to advance public knowledge of human origins science:

- Promote a perspective of paleoanthropology that demonstrates how the research is relevant in all aspects of our lives today (e.g., diet trends and our ability to run marathons)
- Offer targeted information for specific interests (e.g., mothering and childbirth)
- Link our past history with our future survival (e.g., our evolutionary history demonstrates that we are part of the biological world and are susceptible to issues that face all species)

All of the participants expressed strong support for ongoing meetings and to include additional institutions with mission compatibility. Everyone appreciated the opportunity to convene in person, to share ideas and become more familiar with each organization’s mission, processes and even challenges.

All who attended (and even those who could not be present) shared their deep appreciation for Life Trustee Joan Travis, who sponsored this inaugural Leadership Summit. Having first met Louis Leakey in 1965, she has been a patron of paleoanthropological research for nearly 50 years. Mrs. Travis immediately recognized the benefits that this type of gathering can have and believes that if productive relationships are built, positive outcomes will be reached. We are grateful for Mrs. Travis’ enthusiastic support of the Leadership Summit.

In conclusion, the Leadership Summit was a success and has set the stage for future collaborations. The discussions and ideas generated will continue to evolve and shape the future of human origins research.

The organizers wish to extend a special thank you to the sponsors, participants, and everyone who contributed to the success of the Summit.
Grantee Spotlight: Moreen Uwimbabazi

H. GREGORY, GRANTS ASSOCIATE

THIS SPRING The Leakey Foundation awarded Baldwin Fellowships to five outstanding grantees, including Moreen Uwimbabazi, who is currently pursuing her Ph.D. in Zoology at Makerere University in Uganda. The Baldwin Fellowship is a unique program that helps to provide scholars from developing countries the opportunity to study abroad, and so in May 2015 Uwimbabazi will be visiting Harvard University to continue her research for the remainder of the year.

Currently Uwimbabazi is collecting field data on the habituated chimpanzee community at the Kanyawara study site in Kibale National Park in Western Uganda. This is the first phase of data collection for her project entitled, Nutritional ecology of female chimpanzees: Relationship between macronutrient intake, energetic condition, and growth, reproduction. She is in the process of recording feeding rates of individual female chimpanzees at different reproductive states and growth stages. These feeding rates are obtained by counting how many food items are placed in the mouth during multiple one-minute periods. Uwimbabazi is also doing basic processing of food item samples, which she will run through near-infrared spectroscopy (NIRS) at Harvard University.

So how are things going in the field? Uwimbabazi reports, “A good day for me is when I go out in the morning and choose a female individual to follow, and I am able to get at least 20 feeding rates without losing this individual.” However, this is not always the case. There have been months when the chimpanzees spend the whole day feeding in thick undergrowth, making data acquisition difficult, and in times of food scarcity, she often encounters groups of only males. Long treks and elephant encounters also add to the difficulty. Uwimbabazi isn’t discouraged, and she tells us that her advisors, field assistants and the outgoing field manager of the Kibale Chimpanzee Project (KCP) have been quite helpful in this phase of data collection.

Uwimbabazi’s upcoming visit to Harvard, under the guidance of host sponsor Richard Wrangham, will be a busy time. Besides attending class, she will learn how to run her samples through NIRS. In addition, she will begin analyzing the KCP long-term database, which includes minute-by-minute data on food selection by individual females conducted over 5 years.

Finally, Uwimbabazi be coached on how to apply the Geometric Framework (GF) to her data. She explains, “The GF will basically show how female chimpanzees prioritize some nutrients while regulating others. GF is a state-space modeling approach in which the daily intake of nutrients and non-nutrient items (i.e. indigestible fiber) is plotted in graphical space with each axis representing a different nutrient item in the diet. From this, I will observe which parameters female chimpanzees choose to vary when faced with variable food choices and which diet components are maintained or prioritized.”

In addition to her research, Uwimbabazi is currently the deputy field manager for KCP. In 2016 she will take over as field manager. She plans to continue in this position after she graduates and hopes to carry out further research on primate nutrition.

We wish Uwimbabazi the very best in her future endeavors and give a special thanks to Life Trustee Joan Donner, and Trustees Mark Jordan, Naoma Tate and Bill Wirthlin for their generous support of this Baldwin Fellow.

To learn more about the Baldwin Fellowship visit leakyfoundation.org/baldwin
In Memoriam: Robert M. Beck

ROBERT M. BECK passed away on May 9, 2014, after a long struggle with Parkinson's disease. Robert was a founding board member of The Leakey Foundation and his philanthropic support in the formative years of the Foundation was instrumental in making the organization what it is today.

In 1970, Robert Beck made a challenge gift of one million dollars to encourage others to share his commitment to this fledgling cause. A profile of Robert in a 1977 issue of AnthroQuest stated, "...his gift carried with it a philosophy that has been basic to Robert Beck's thinking throughout his life: Explore the human element. If the individual is given a chance to apply his own resources and exclusive talent for imagination and creativity, mankind reaps the greatest harvest." Louis Leakey hailed the gift as "opening a window towards man's knowledge of himself."

Robert's life was filled with exceptional accomplishments, from pioneering work in early computing to La Vigne Organics, the thriving biodynamic and organic farm that he started in 1994 with his wife Helene. Robert was a cattle rancher in Montana for more than 30 years, where he and Helene initiated many projects aimed at the protection of the land and conservation of its resources. Robert was also a founding board member of the Institute of Human Origins. Robert Beck's memory is to be cherished, and his life to be celebrated.

In Memoriam: Frank M. Woods

FRANK M. WOODS passed away on May 8, 2014, at the age of 81. He served on The Leakey Foundation Board for many years and was later honored in 2005 as a Life Trustee.

Frank was an active member of the Bohemian Club, the Pacific-Union Club, the California Tennis Club, the Guardsmen, and the San Francisco Golf Club. He became an advocate for the American wine industry, especially Sonoma County where he co-founded the award winning Clos du Bois Winery. Over the years he was chairman of the Wine Institute and chairman of the American participation in the International Organization of Viticulturalists (OIV). He served on the boards of the Fine Arts Museums of San Francisco and the Young Audiences of San Francisco.

Frank is survived by his beloved wife, Kay Harrigan Woods (Leakey Foundation President 1992-2004 and Life Trustee), along with three children and their spouses, six grandchildren, and Frank's brother and sister. Frank was a brilliant entrepreneur, a true Renaissance man, and a dear friend. His leadership and camaraderie will be deeply missed.
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