Ancient DNA

Sex and the CTAG: what ancient DNA tells us about our ancestors’ liaisons

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The development of next-generation sequencing (NGS), a technology coincidentally well-suited to highly fragmented, low copy number DNA sources, spawned a rapid expansion in the field of ancient DNA (aDNA). It has gathered a reputation as a sexy subject, quite literally. Some of the headlines targeted to the public: ‘Mystery humans spiced up ancients’ sex lives’ in *Nature News* or ‘Viking sex tourists lived happily ever after with Britons’ from *The Independent,* would make any scientist blush and probably want to bang their head against a brick wall. As problematic as these headlines are, people keep writing them because sex sells and while aDNA might not tell us exactly what our ancestors were into, it has and will continue to provide other unique insights regarding our reproductive past.

What aDNA can tell us about actual ancient sex

The bread and butter of the aDNA world is the human migration and admixture story. In 2010, the first draft genome of a Neanderthal was published and with it the first concrete evidence that as anatomically modern humans left Africa, they met and had children with their Neanderthal cousins already inhabiting wide swaths of Eurasia. The genome of another sister species from a cave in the Altai Mountains, the Denisovans, led to the re-discovery that when humans meet each other, they have sex. Fragments of this archaic hominin’s genome are found today in high percentages (~6%) in Australasian and Oceanic populations and specific genes from this ancestral population have conferred an adaptive advantage to high altitude in Tibetan people today. Not only have humans had conjugal relations with their archaic hominin partners, but the Neanderthals and Denisovans themselves had offspring when they met, as evidenced by one incredible find from the same cave. From a single fragment of loose bone, identified through its uniquely hominin protein structure, researchers at the Max Planck Institute in Leipzig extracted aDNA and discovered that the bone belonged to the child of a Neanderthal mother and Denisovan father.
In more recent history, aDNA studies on all continents and time periods have shown that at any given point, different populations of people have met and then have had sex with each other, producing the populations in those areas today. For example: in Europe, hunter-gatherer populations (whose ancestors had sex with Neanderthals), had sex with incoming Neolithic farmers from Anatolia and their offspring then later had sex with the people coming in from the Eurasian Steppe. Today's reality of mass migration into Europe and melting-pot culture is nothing new and if aDNA has taught us anything, it is that the sharing of genes and culture between previously separated populations is the norm (and will likely benefit our descendants too).

**Swipe left, swipe right**

Sexual selection is a key component of evolution and modern studies indicate that visual and non-visual cues (e.g., pheromones) affect our mate choice. What made cross-species coupling so attractive? Well, we don't really know as, after all, beauty is in the eye of the beholder. Neanderthals have been physically reconstructed from skeletons using forensic techniques, but what about the Denisovans, where we have only a genome? Researchers have used ancient bone epigenetic information to attempt a reconstruction and ancient protein identification will eventually reveal the Denisovan skeletons hiding in our museum collections. As for the non-visual aspect of attraction, it has been shown that humans tend to choose partners with innate immunity genes as different as possible from their own, which confers an advantage to their children. This phenomenon could very well have been at play in the past because some of the genes we have inherited from Neanderthals that have not been purged by purifying selection, have helped our immune systems. This is a double-edged sword though as nature does not develop an immune response without an infectious trigger.

**If you touch each other, you will get chlamydia... and die**

For anyone who was forced to sit through a sexual education class taught by the reluctant school football coach, the above quote from the 2004 film *Mean Girls* really hit home. Of course, it's exaggerated for the joke and in today's world, even if you do get chlamydia or any other bacterial sexually transmitted infection (STI), you most likely will not die because it can be treated with antibiotics (for now). However, for most of human history, this was not the case and even today viral infections prove more difficult and can have lifelong complications. So, what has aDNA shown us about ancient sexual health?

First, it is important to understand how we get aDNA from deceased humans. It can be sourced from any biological remains including skeletons and mummies; however, skeletons are the primary target for aDNA studies due to the wider availability and being less chemically complicated than mummies. The inorganic matrix of bone is like a sponge for proteins and DNA, adsorbing whatever is near at the time of death. Most studies interested only in the human genome target the *pars petrosa*, or the inner ear canal as this bone is very dense, full of osteocytes (bone cells) and tends to preserve well, leading to high human DNA content. Teeth are also quite dense and thanks to the blood in the pulp chamber, provide a diverse source of human and microbial DNA. Dental calculus, a calcified biofilm that forms on unbrushed teeth, is particularly prevalent in ancient mouths as they did not have access to modern dental care and provides a rich data source for mining the oral microbiome. Enterprising researchers have used aDNA to unravel the mysteries of ancient non-STIs like plague (*Yersinia pestis*), *Salmonella enterica* and Hansen's disease or leprosy (*Mycobacterium leprae*), and some STIs like syphilis (*Treponema pallidum*) and hepatitis B (HBV).

Due to their structure, treponemal diseases and viruses tend to degrade more easily than thick-walled bacteria and are difficult to detect molecularly, especially when copy numbers are low in comparison to the host genome. However, a few rare genomes from 17–19th century congenital syphilis cases have been sequenced, showing that different strains led to similar physiological conditions. A number of HBV genomes from across Eurasia (up to 7000 years old) show high diversity of strains in the past, a high correlation with human migration and a history of recombination. The process of recombination occurs when a host is infected with multiple strains, or as a journalist might sensationalize: ‘multiple sex partners contributed to mutant virus’. In all seriousness, it’s not clear how that recombination event occurred. HBV can be transmitted via any infected bodily fluid and for all we know, our ancestors had some blood-drinking rituals or were sharing tattoo needles. The point is that, as we saw in the previous section, throughout history groups of people have come into contact with each other and not only shared their genes, but also their diseases.

With this in mind, we should expect that not only do Neanderthal genes live on in our genomes, but their STIs should still be in circulation as well. A recent paper noted that the pattern of variation in human papillomavirus (HPV) might indicate host transfer from Neanderthals to modern humans. This hypothesis is hard to pin down however, because the high mutation and recombination rate in these organisms means that a lot of information gets erased and is impossible to see in modern genomes. This is where aDNA could step in to save the day. The
classics, chlamydia and gonorrhoea unfortunately may never be recovered from ancient remains because they infect soft tissues that are not in direct association with bones; however, if an oral infection was present there is hope that it could be found in teeth. One STI that we should find in teeth is herpes simplex virus 1 (HSV1), which infects over 60% of the global adult population and HSV2 anywhere between 10 and 25% (and can infect the oral cavity in some cases). Considering the fact that teeth are a popular target for aDNA studies, HSV1 lies latent in the trigeminal ganglia and has been recovered from dental plaque and teeth in studies of periodontal disease, one would think that nearly every skeleton should have some HSV1 DNA waiting to be discovered. Looking at the current aDNA pathogen literature, this does not seem to be the case. Why? Perhaps because HSV1 tends to stay latent most of our lives and would only lead to high viremic levels in rare circumstances or maybe our current methods and sample sources are not ideal for detection.

For this reason, our lab is trying additional biologically techniques to beef up detection rates. Proteins, with more complex structures, tend to preserve better than DNA fragments and could be present even when DNA is not detectable. Unique peptides, such as those found in the HSV1 viral capsid or HBV external core antigens could be used to detect pathogens and in the latter case even identify the subtype in the absence of the high DNA coverage needed to determine strains. These ancient proteins could even do more than help determine infections; they might help us identify the other potential side effect of sex: pregnancy.

Ancient proteomics could expand our understanding of historical circumstances in pregnancy and the mortality surrounding it. Until very recently, humans did not have the benefits of advanced surgical knowledge, blood transfusions or antibiotics. Thus, we would expect there to be a much higher number of women who died during and around childbirth than today and at least some of the skeletons we are analysing should fall into this category. Fetal bones do not preserve well and can be missed during excavation leading to an under-representation in

Don’t have sex, because you will get pregnant and die

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the archaeological record. There is no way to tell just by looking at a female skeleton whether she was pregnant, but many proteins are produced in association with pregnancy including human chorionic gonadotropin, which is not produced in other physiological states (except testicular cancer, but here I think it is easy to distinguish between this and pregnancy). As the field expands to include new wet lab techniques and our online reference databases continue to grow, we can expect to learn a lot more about our sexual history.

Summary

We will never know whether Neanderthal women gossiped with their girlfriends after an encounter with an anatomically modern human, but we do know that certain genes have been preferentially conserved in modern genomes, some with obvious adaptive benefits and it appears that the Neanderthal male–Homo sapiens female pairings were more evolutionarily successful than the other way around. We know that they gave us their diseases, but also their immunity genes. The recovery and analysis of aDNA and proteins has revealed two underlying truths about human nature: 1) wherever and whenever humans meet, they will hook up and 2) humans love talking about it.

Further reading

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