ANCIENT BIOMOLECULES INITIATIVE

Molecular Signatures from the Past

Sacrificial llamas and DNA

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The ancient remains of sacrificial llamas and alpacas in South America may provide clues about the history of their domestication, and why the quality of their valuable wool seems to have been much greater before the Spanish conquest.

Helen Stanley (left) and Jane Wheeler (right) examine the mummified remains of a sacrificial llama at the pre-Inca site of El Yaral in Peru

South American camelids (the New World relatives of one- and two-humped camels) are fascinating animals. With two wild species (vicuña and guanaco) and two domestic types (llama and alpaca), these animals are a valuable part of South American biodiversity. Their wool provides vital income for the poorest sector of the population and vicuña has the world's most valuable fibre.

We wanted to know more about genetic variability in living camelids and investigate the relationships of the domestic types to their wild ancestors. This information will help to plan conservation and breeding programmes for these important animals.

Recent advances in DNA technology and Andean archaeozoology mean that we can now investigate these questions in a way that was not previously possible. By analysing ancient and modern DNA, we found that there are two distinct lineages of camelids, which highlights the genetic distinctiveness of the wild vicuña and guanaco. Surprisingly, the domesticated camelids could belong to either lineage, suggesting that they had come from hybrid stock.

The wool from buried pre-Spanish llamas and alpacas preserved in hot desert conditions has finer fibres than anything known today. Were these animals pure-bred or were they specialised breeds, lost during the devastation of the conquest? The answer to this question may suggest breeding programmes to improve the quality of modern camelid wool.

We studied archaeological specimens ranging from 8,500 years old (before domestication began) to 400 years old, and compared them with modern camelids. In the process we are finding out more about how DNA is preserved in archaeological specimens.

We were particularly interested in some 1,000-year-old llamas and alpacas that had been sacrificed and buried under house floors, where they became naturally mummified in the dry environment.

We were disappointed to find that these extremely well preserved specimens did not contain well preserved DNA, maybe because of the fluctuating temperature that they had been exposed to over time.

Domesticated llamas in the High Andes. Most of these animals are held by traditional Andean pastoralists, with llamas used as pack animals and providing fibre, meat and fuel.

The Ancient Biomolecules Initiative is a five-year programme to understand the fate of biological molecules in archaeological and fossil materials, and to explore the applications of this new knowledge. The Initiative is funded by the Natural Environment Research Council.

Natural Environment Research Council

Sacrificial llamas and DNA: the science in detail

Archaeozoology and molecular genetics can make important contributions to our understanding of animal and plant domestication. Andean civilization was non-literate, so knowledge of pre-Spanish llama and alpaca breeding practices must be reconstructed from archaeological remains. For example, the recent discovery of 900-1,000 year old naturally mummified llamas and alpacas provides a unique source of information on Andean breeds before European contact.

Our aim is to identify camelid genotypes, at various different time points, to provide important new information on the evolutionary relationships of these animals.

Methods

To investigate genetic differences between wild and domestic camelids, and historical and modern samples, we studied mitochondrial DNA sequences using the polymerase chain reaction (PCR) to copy DNA fragments, either ancient or modern.

Mitochondrial DNA has a high mutation rate, allowing us to detect differences between closely related animals. Certain regions of DNA evolve at different rates, with the D-loop (or control region) being relatively fast, the cytochrome b gene slower and 16S rRNA slower still.

We investigate changes over time (or space) by looking at variations in DNA sequence: the positions of these variable sites are first determined in modern specimens. This information is used to design appropriate PCR primers for ancient specimens. Almost any combination of PCR primers can be chosen to amplify modern DNA but for ancient DNA, which is usually fragmented, primers which amplify short fragments (eg 80 - 300 base pairs long) are needed. We designed camelid-specific D-loop primers to prevent contamination by humans, or by species studied routinely in our laboratory.

Modern camelids

We surveyed variations in D-loop DNA sequences of 129 extant south American camelids. By including samples from all phenotypes of the four species/breeds and covering a broad geographic range, these

The pre-Inca site of El Yaral (AD 950 - 1350) at an elevation of 1,000 metres in the extremely arid coastal desert of southern Peru. Compacted floors of fine gravel, sand and clay covered these shallow burial holes containing sacrificial alpacas and llamas, together with offerings such as guinea-pigs, coca leaves and shells.

data provide a framework of variation among living animals against which results from ancient specimens can be compared. We identified 43 variable sites in the D-loop, which define over 40 genotypes (sequence divergence from 0.3 - 7%). Phylogenetic analysis of these data identifies two distinct lineages which highlight the genetic distinctiveness of the wild vicuña and guanaco and the hybrid nature of the domestic llama and alpaca. More detailed analysis of these data is continuing.

Ancient DNA

We were unable to get reliable DNA amplification from the cytochrome b gene and D-loop mitochondrial DNA in ancient specimens, but we have sequenced part of the mitochondrial 16S rRNA gene in pre-Spanish llamas (see figure below).

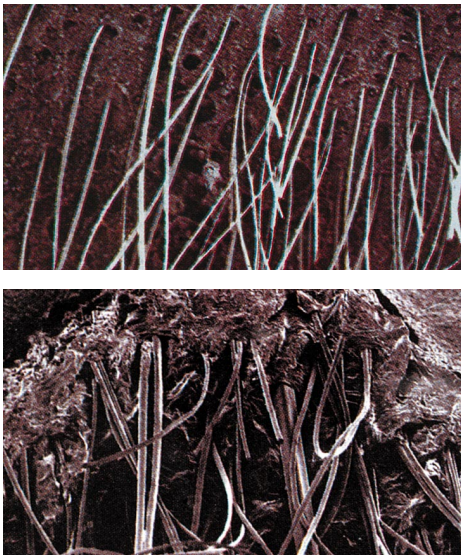
It may be that certain regions of the mitochondrial genome are more susceptible to degradation than others and/or that the 'sensitivity' of primer pairs varies (H Poinar, pers. comm): we discovered that the 16S primers gave the highest success rate compared to any camelid-specific primer pair tested.

Partial Sequence of mitochondrial 16S rRNA gene from El Yaral specimens. The sequence of the 16S rRNA gene from 1,000-year-old llamas is very similar to that of modern animals, as we would have expected. Sequences below are from two modern samples: Arabian camel (Milinkovitch et al 1994) and alpaca, and from two of the 1,000 year old desiccated llamas from El Yaral (labelled 274 and 231). The camelid sequences are from cloned fragments of the 16S gene.

The future

The camelid project is continuing (funded by the Darwin Initiative) with an emphasis on nuclear DNA (microsatellite) markers to further assess the distribution of genetic variation in vicuña and guanaco populations. These markers will also be useful for further analysis of the wild/domestic animal relationship question.

By providing genetic data, our research has major implications for contemporary breeding and conservation policy for all four South American camelids.



Pre-Spanish specimens exhibit a uniformity of wool colour, distribution and fineness which is absent in contemporary animals. The top photograph reveals the homogeneity of the llama fibre from 1,000 year old mummy sample compared with the uneven quality of today's individuals (below). The fibre of the llamas found at El Yaral is not only finer than that in contemporary llamas but is also finer than some alpacas.

Llama from El Yaral site. This animal's body has been preserved by natural desiccation. It is approximately 1,000 years old.

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Arabian camel   CAGAAAAACCTCCGAGTGACTAAAATCTAGATTTACCAATCAAAATGTAGTGTCACTTATTGATCCAAAAT-ATTTGATCAACGGAACAAG
Extant alpaca   GAACAGAAAAACCTCCGAGTGACTAAAATTTAGATNTGCCGATCAAAATGTAGTGTCATTTATTGATCCAAAGTTATTTGATCNACGGNACA
Llama 274        AACAGAAAAACCTCCGAGTGACTAAAATTTAGATCTGCCGATCAAAATGTAATGTCACTTATTGATCCAAAGTTATTTGATCAACGGAAACA
Llama 231       AAACAGAAAAACCTCCGAGTGACTAAAATTTAGATCTGCCGATCAAAATGTAGTGTCATTTATTGATCCAAAGTTATTTGATCAACGGAAACA
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