PART III

BACTRIAN CAMELS

(Camelus bactrianus)

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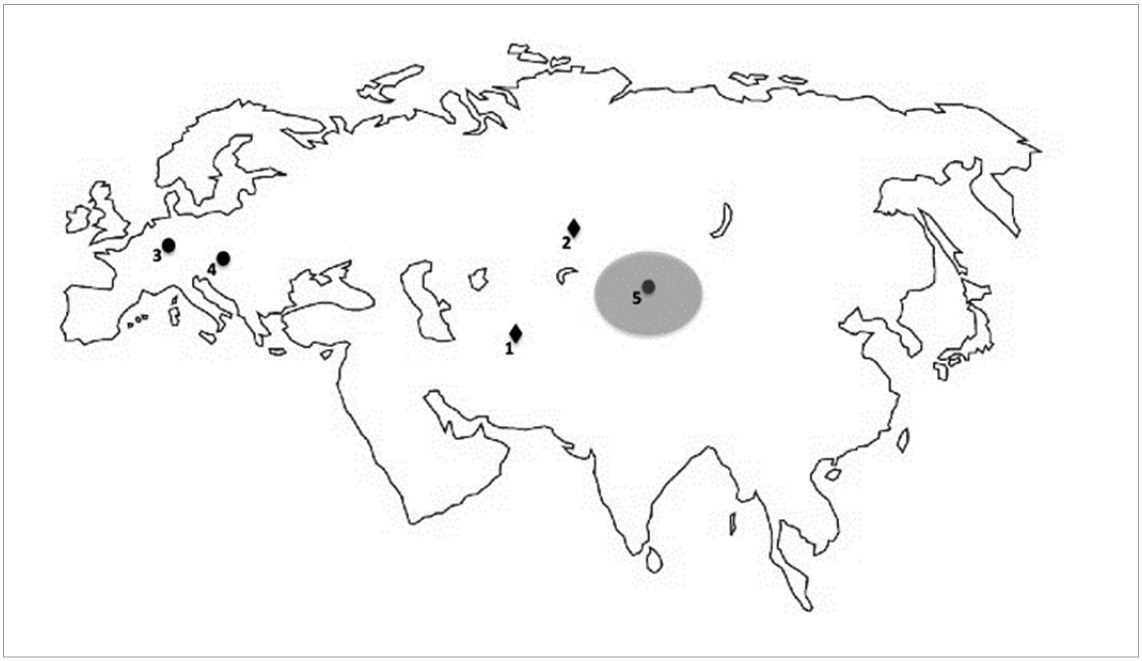
Ancient DNA Reveals Domestication Process: The Case of the Two-Humped Camel

The domestication of the two-humped camel (*Camelus bactrianus*) promoted remarkable progress in cultural and economic development for ancient human civilizations in the steppes of Eurasia. However, the evolutionary relationship between domesticated and the extant wild two-humped camels (*Camelus ferus*) as well as time, place and motivations for domestication of these animals remain unresolved.

Here, a particular fragment of the mitochondrial DNA (458bp hypervariable fragment of the control region) was analyzed in 12 bone samples of Bactrian camels. The bone material was collected from Late Bronze and Early Iron Age sites in Siberia and Uzbekistan. Subsequently, ancient DNA sequences from these samples were compared to DNA sequences from 122 modern domestic Bactrian camels from China and Mongolia, eight modern domestic Bactrian camels from German and Austrian zoos and 20 modern wild camels from Mongolia (see graph 15).

Phylogenetic distances between wild and domestic populations is inconsistent with the hypothesis that the progenitors of the wild *Camelus ferus* population were ancestral both to the prehistoric the modern domestic camels. Low genetic diversity observed in all domestic populations supports the idea of a single camel domestication center, possibly in the western part of Central Asia, and indicates that it is very unlikely that the lineage of the extant wild two-humped camel is the progenitor of the domestic Bactrian camel.



Graph 15: Overview of the origin of the samples analyzed:
1 Late Bronze and Early Iron Age samples from Bandixon, south Uzbekistan
2 Late Bronze Age samples from Om‘1, West-Siberia
3 Modern domesticated camel samples from zoos, Frankfurt/Germany
4 Modern domesticated camel samples from a zoo, Vienna/Austria
5 Modern domesticated and wild camel samples from the borderland of China and Mongolia

INTRODUCTION: THE QUESTION OF ANCESTRY IN CAMEL DOMESTICATION

Domestication of the two-humped camel facilitated the cultural and economic development of prehistoric human civilizations in the Eurasian dry zones. The Bactrian camel continues to be used today by modern rural and nomadic societies as a source of transportation, labor, meat, milk and wool. Camel dung replaces scarce firewood as fuel in the mostly treeless steppes. Despite the great economic impact of domestic camels for the inhabitants of the Central Asian deserts, the time and place of domestication remain uncertain (Benecke 1994).

The only surviving wild camel species, *Camelus ferus*, is commonly thought to be the progenitor of the domestic Bactrian camel. As we will show, however, modern investigation techniques such as ancient DNA sequencing make it possible to draw a different conclusion. During the Pleistocene, wild camels were distributed throughout the desert areas of Eurasia, but their numbers decreased over time (Benecke 1994, Peters/von den Driesch 1997, Peters 1998). The westward extent of the ancient wild camel population distribution is unknown, because of the paucity of skeletal evidence and the small number of residual modern populations restricted to four separate desert areas in China and Mongolia (Bannikov 1976, Hare 1997, Mix et al. 1997). The worryingly low number of wild camels at present makes it impossible to reconstruct their original zoogeographic distribution, particularly at the time of domestication. Additionally, incomplete knowledge about the range and variability of the wild camel population at the onset of the domestication process makes it difficult to determine the proceedings of camel domestication.

THE QUESTION OF LOCATION IN CAMEL DOMESTICATION

Archeological records show evidence of a closer relationship between humans and the Bactrian camel beginning in the late 4th and early 3rd millennium BCE (Bulliet 1975, Benecke 1994). The earliest skeletal remains come from sites in Anau and Geoksyur at the Kopet-Dagh foothills in the borderland between Turkmenistan and Iran (Kuzmina 2008). A similar situation appears at the northern edge of the Kopet-Dagh mountains, where archeological layers at Shor- and Khapuz Depe produce finds of the Bactrian camel dating to the early 3rd millennium (Compagnoni/Tosi 1978). Further, camel bones dating between 2,500 and 2,200 BCE originate from the Turkmen settlements of Altyn Depe, Namazga Depe and Kelleli (Peters/von den Driesch 1997, Kuzmina 2008). At the Shahr-i-Soktha site in south-east Iran, finds of collected camel hair and dung date to the first half of the 3rd millennium BCE. This suggests the presence of animals that lived in close association with the inhabitants of the settlement and very likely represent domesticates (Peters/von den Driesch 1997). Based on the provenience of the finds at Shahr-i-Soktha in south-east Iran, excavators have assumed that camel husbandry for meat, wool and dung production has been practiced in this region since the Bronze Age (Köhler 1981). Likewise, clay figures of cart-drawing camels of the same period from Ulug Depe imply a use of domesticated camels as draught animals (Kuzmina 2008). The presence of camel bones in Bronze Age strata from sites in Iran and southern Turkmenistan suggests that people of the Iranian Plateau and the Kopet-Dagh foothills area played a major role in the domestication process of the two-humped camel (Benecke 1994).

Contrary to this hypothesis, some archeologists and zoologists do not believe that paleontological and archeological evidence from south-western Central Asia can be interpreted as an indication for an ongoing process of domestication, as they see no proof of the presence of the wild *Camelus ferus* during early to middle Holocene times in this area. The authors argue that the center of domestication must lie further east, in areas where people were acquainted with the wild form over an extended period of time, for example in southern Kazakhstan or northwestern Mongolia (Peters/von den Driesch 1997). The sudden appearance of camel bones in Turkmen and Iranian archeological layers dated to the Bronze Age, as well as missing skeletal remains of wild camels in earlier Neolithic strata in these regions convince them that people from the Kopet-Dagh foothills acquired the fully domesticated two-humped camels from eastern Asia.

However, differentiation between wild and domestic camel bone material is difficult and the earliest evidence of fully domesticated camels from southern Kazakhstan and north-western Mongolia are only dated to the 2nd millennium BCE (Köhler 1981, Peters/von den Driesch 1997, Mukhareva 2007).

THE VALUE OF ANCIENT CAMEL DNA: MATERIAL AND METHODS

To further examine the origin of the domestic Bactrian camel, we investigated mitochondrial DNA from prehistoric camel bones and compared them to extant wild and domestic Asian camels.

Archeological bone material of *Camelus bactrianus* was collected from six different Late Bronze and Early Iron Age sites (dating 1500 BCE–100 CE) in western Siberia (*Rayon Kujbyšev, Province Novosibirsk*) and southern Uzbekistan (*Rayon Bandixon, Province Suchandar'ja*). Further information about the samples is given in table 6.

Lab Code	**Archeological Site**	**Dating**
Om'1.1	Om'1, Novosibirsk/West-Sibiria (Settlement)	Late Bronze Age (1500–800 BC)
Ban 1	Bektepa (Bandixon II), Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (4th century BC)
Ban 2	Bektepa (Bandixon II), Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (4th century BC)
Ban 3	Bektepa (Bandixon II), Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (4th century BC)
Ban 5	Bektepa (Bandixon II), Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (4th century BC)
Ban 6	Jalangtushtepa, Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (3rd century BC – 1st century AD)
Ban 7	Kurgansol, Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (4th – 2nd century BC)
Ban 8	Kindyktepa, Bandixon/South-Uzbekistan (Settlement)	Early Iron Age (4th century BC)
Ban 9	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 10	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 11	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 12	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 13	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 14	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)

Lab Code	Archeological Site	Dating
Ban 15	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 16	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)
Ban 17	Majdatepa (Bandixon I), South-Bandixon/Uzbekistan (Settlement)	Late Bronze Age (1400–1000 BC)

Table 6: Archeological samples.

For the specifically interested reader we shall explain the procedure of ancient DNA extraction in more detail. Two separate bone samples were cut from each individual and then powdered. Extraction of ancient DNA was carried out by incubation of 0.5 g to 2 g powdered bone samples in 3 ml to 12 ml extraction buffer (2.5 ml to 10 ml 0.5 M EDTA, pH 8; 0.25 ml to 1 ml 5% N-lauryl sarcosine; 30 µl to 120 µl 20 mg/ml Proteinase K) on a rotary shaker overnight at 37°C. Subsequently the DNA was extracted using one volume phenol/chloroform/isoamyl alcohol (25:24:1); the supernatant was concentrated by Amicon Ultra 15 (50 kDa) dialysis and finally washed several times with UV-treated HPLC-grade water.

Primer pairs for six overlapping fragments of the camelid mtDNA were designed to cover the first 458 bp of the control region (np 15436–15894; numbering as per Cui et al. 2007). Because of the poor preservation state of some of the bone samples, six additional primer pairs amplifying smaller, non-overlapping fragments were created. All likely non-variable base positions between the non-overlapping fragments were filled up with the reference sequence (NCBI accession number: NC_009629). Further information about primer sequences and primer positions are given in table 7 and 8.

For data analysis, mtDNA sequences were aligned using the MegAlign program from the DNA Star Software package (Version 7.2). The sequences of aDNA-samples were subsequently compared to 122 modern domesticated Bactrian camels from China and Mongolia, to eight modern samples of domesticated camels from German and Austrian zoos, and to 20 modern wild camels (*C. ferus*) from Mongolia (totaling 162 sequences). A reduced median-joining network was constructed using the Network 4.5.1.0 program (Fluxus Technology, Clare, Suffolk, UK; http://www.fluxus-technology.com).

System	Primer	Sequence (5'-3')	Primer Position	Product Position	Product with/ without Primer
1	U1	ggaattctcattaaactacccctgac	15409–15435	15436–15525	137/91
	L1	gcaacgcgtgctgtgacat	15526–15544		
2	U2	tgcccaacgtgcatgaaacttc	15495–15516	15517–15610	144/95
	L2	atttgacataatgtgctatgcacgaac	15611–15637		
3	U3	atcgtgcataaatttgtttgccc	15558–15580	15581–15671	138/92
	L3	ggcctggtgattaagctcgtgat	15672–15694		
4	U4	catttccagtcagtacgcatatcataac	15638–15665	15664–15745	133/81
	L4	gtttagaaacccccacaatggatg	15746–15769		
5	U5	tcatcaacccgctcagcag	15703–15721	15720–15824	148/104
	L5	tgtcttatttaagaggaaagtgtgg	15825–15849		
6	U6	ctttatcaggcatctggttcttacttc	15749–15775	15774–15894	177/120
	L6	aattataaaagtaccaaatgcatgacacca	15895–15924		

Table 7: Overlapping primer pairs for mitochondrial DNA analysis.

System	Primer	Sequence (5'-3')	Primer Position	Product Position	Reference-sequence	Product with/ without Primer
1	BanU1	ggaattctcattaaactacccctgac	15409–15435	15436–15499		117/65
	BanL1	tgtcagtattgaagtttcatgcacgtt	15500–15526		15500–15522	
2	BanU1b	cccaacgtgcatgaaacttcaatact	15497–15522	15523–15563		90/42
	BanL1b	atgcatggggcaaacaaatttatg	15564–15587		15564–15585	
3	BanU2	cataaatttgtttgccccatgc	15564–15585	15586–15618		81/34
	BanL2	tggaaatgatttgacataatgtgctat	15619–15645		15619–15625	
4	BanU3	tacatcttattcttgttcgtgcatagc	15599–15625	15626–15680		100/64
	BanL3	cacgcggcctggtgattaa	15681–15699		15681–15769	
5	BanU4	atccattgtgggggtttctaaac	15747–15769	15770–15792		70/24
	BanL4	taggtgagatggtcctgaagtaaga	15793–15817		15793–15855	
6	BanU5	cacactttcctcttaaataagacatctcga	15826–15855	15856–15887		89/33
	BanL5	agtaccaaatgcatgacaccacagttat	15888–15915			

Table 8: Non-overlapping primer pairs.

AUTHENTICITY OF ANCIENT DNA SEQUENCES

The most serious problem with ancient DNA work is contamination, which can occur within each step of ancient DNA analysis: in the phase of sample preparation, during extraction and PCR-set up, or during the amplification step.

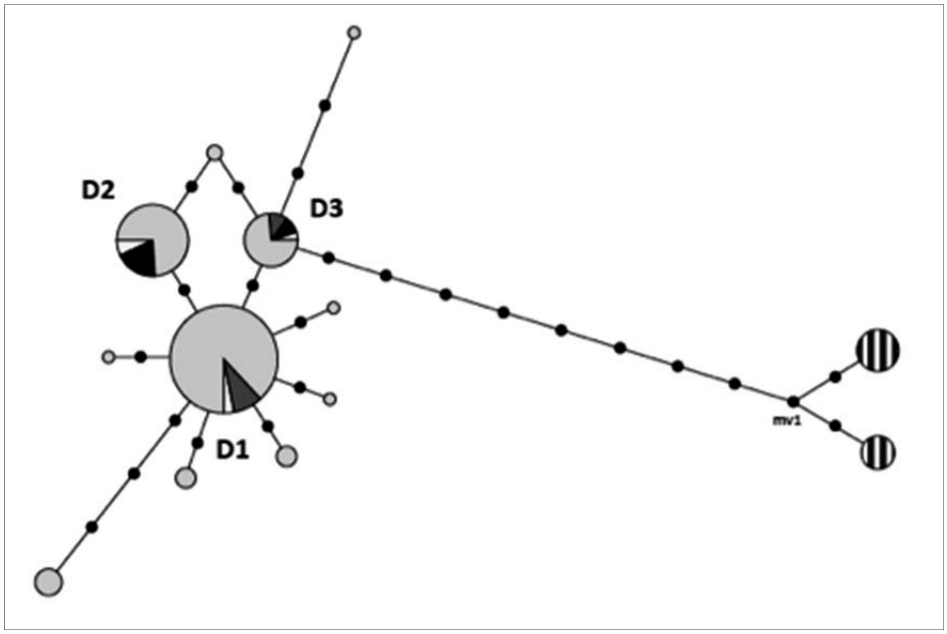
Ancient DNA is present in only small amounts, is seriously damaged and difficult to amplify. In contrast, modern DNA is ubiquitous, predominates over ancient DNA in quality and quantity, and is therefore easier to amplify. Due to the nature and degradation properties of ancient DNA, strict measures have to be applied to prevent sample contamination and to ensure the generation of authentic and meaningful data.

The ancient DNA work described above was carried out in the laboratories of the Institute of Anthropology at the University of Mainz, Germany. To avoid contamination in our study, all pre-PCR working steps including sample preparation, extraction and PCR-set up were conducted in a clean-room laboratory free of molecular work. All the following steps, including amplification and sequencing, were performed in a separate post-PCR laboratory.

Two separate bone samples were obtained from each individual and analyzed independently. In addition, specific primers were designed so that they only amplify Bactrian camel DNA. Various blank controls carried out along with both the extraction and PCR-reaction steps were always negative and did not contain any DNA. 12 out of 17 bone samples yielded DNA and gave reproducible results.

RESULTS: DIFFERENTIATION BETWEEN PREHISTORIC AND MODERN BACTRIAN CAMELS

DNA sequences between wild and domestic camels showed significant differences. To illustrate these differences and the relationship within and between the different wild and domestic camel populations, a median-joining network (see figure 2) was generated, using the Network 4.5.1.0 program (Bandelt et al. 1995). The network contains a total of 162 sequences. The size of the circles in the network is consistent with the number of samples with a particular DNA sequence, which thus carry the same mitochondrial haplotype[1].



Graph 16: Median-joining network:
light grey = modern domestic camels; black: modern zoo samples;
dark grey = Late Bronze Age;
white = Early Iron Age;
black/white cross-stripes = mitochondrial Genotype of the wild camel;
small black dots = nucleotide changes between samples, caused by mutations.

In graph 16, sequences of the modern domestic camels are represented in light grey, modern zoo samples are marked in black, dark grey segments are from the Late Bronze Age, and Early Iron Age samples are labeled in white. Haplotypes of the wild camel are depicted in black/white cross-stripes.

Nucleotide changes between samples, caused by mutations, are reflected by small black dots. Distribution of the mtDNA-sequences within the network shows a great homogeneity between all domestic Bactrian camel populations, whereas all ancient samples cluster with modern domestic camel haplotypes. The samples (listed in table 6) Ban 3, Ban 10, Ban 12, Ban 13, Ban 14, Ban 15 and Ban 17 group in cluster "D1", while samples Om'1 and Ban 5 group in cluster "D2" and Ban 1, Ban 9 as well as Ban 11 group in cluster "D3".

Within the domestic camel populations, haplotypes differ from each other mostly in one or two transitions. Furthermore, the star-like pattern of modern *Camelus bactrianus* haplotypes based on cluster "D1" may indicate a recent population expansion. By contrast, the sequences of the extant wild *Camelus ferus* can be distinguished into only two haplotypes, which differ from each other though two mutations, namely by a transition and by an indel (insertion/deletion).

[1] As the mitochondrial DNA is only inherited from the mother, we also refer to a mitochondrial genotype as a haplotype.

The phylogenetic network clearly demonstrates the significant difference between the Mongolian wild camel lineages and all domestic lineages, pointing to a long separate population history. This is inconsistent with the idea of the domestication of Bactrian camels from an ancestral *Camelus ferus* population.

CONCLUSIONS

Mitochondrial DNA comparison of all domestic camels (Late Bronze and Early Iron Age from Siberia and Uzbekistan as well as modern camels from China and Mongolia) revealed relatively few nucleotide differences. Considering this low diversity, especially within the ancient samples, a single domestication center seems more likely than multiple origins of domestic camels. Unfortunately, our sample size is small and skeletons mostly stem from adjacent sites in south Uzbekistan. It is thus very likely that we underestimate the actual diversity in early domestic camels. However, it is noteworthy that one of our archeological samples, which comes from a site in Siberia that is approximately 2,500 km distant from the others and dates to the Late Bronze Age, carries the same haplotype as an Early Iron Age sample from Uzbekistan. This at least adds to the picture of limited diversity in early domestic Bactrian camels.

Wild camel sequences are phylogenetically extremely distant from domestic two-humped camels, pointing to a population split long before domestication. Based on modern sequences of the whole mitochondrial genome, Ji et al. (2009) estimated the most recent common ancestor between *Camelus bactrianus* and *Camelus ferus* as being up to 700,000 years ago.

The ancient DNA sequences presented here demonstrate the high similarity between earliest domestic camel populations and modern Asian and zoo camels. The observed difference between the ancient domesticated camels and living wild camels, however, is inconsistent with the hypothesis that the progenitors of the wild *Camelus ferus* population were ancestral both to the prehistoric and recent Bactrian camels. As the extant wild two-humped camel contributed little or nothing to the domestic camel populations, another as yet unidentified wild camel population must be the ancestor of the domestic two-humped camel.

The distribution area of *Camelus ferus* during the early and middle Holocene included the East Asian steppes as far as modern Mongolia and southern Kazakhstan. We consider the sympatric, i.e. overlapping existence of another genetically distinct wild camel population in this region to be very unlikely. In the search for the ancestral wild camel population we thus assume a more western origin of the ancestral Bactrian camel population. As mentioned above, the earliest archeological records of camel bones, cart-drawing clay figures and finds of camel hair and dung come from settlements of the Kopet-Dagh foothills in the borderland between Turkmenistan and Iran and date to the second half of the 4th and the beginning 3rd millennium BCE. In contrast, the earliest evidence of fully domesticated camels in Kazakhstan and Mongolia occurs much later, in the 2nd millennium BCE.

Considering the archeological record, there is no doubt that camel husbandry gained importance in Eurasia during the Bronze Age. As a result of this palaeogenetic study, we propose that the domestication of the two-humped camel took place as a single domestication process, rather in south-western parts of Central Asia than in Mongolia or even East Asia, whereas the lineage of the extant wild *Camelus ferus* is neither the ancestor of the ancient domestic camel population analyzed here, nor of the modern domestic two-humped camels.

Although the observed genetic differences are significant, different ancestry scenarios will have to be modeled and tested by serial coalescent simulations to see whether the observed genetic patterns could also have been produced under alternative – *prima vista* less plausible – demographic history models.

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