

Nuclear Gene Indicates Coat-Color Polymorphism in Mammoths

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Because more than 99% of all species that have ever lived on Earth are extinct, the genetic basis of most phenotypic traits that have evolved during life's history cannot be studied directly. Although it is possible to approximately reconstruct the gene sequences of extant species' common ancestors (1), many extinct species represent end points of evolution, and their gene sequences cannot be investigated by using this approach. For late Pleistocene species, ancient DNA techniques should in principle allow analyses of extinct genes. However, despite progress in the analysis of ancient DNA, to date only short fragments of nuclear genes could be amplified from Pleistocene specimens (2, 3).

We have combined two methods recently introduced in ancient DNA research, large-scale extraction and multiplex polymerase chain reaction (PCR), to amplify 1236 base pairs (bp), including the complete coding sequence, of the single-exon nuclear melanocortin type 1 receptor (*Mclr*) gene from an ~43,000-year-old mammoth (*Mammuthus primigenius*) bone from Siberia. The product of the *Mclr* gene is a key protein in the determination of hair color in both humans and other animals (4), providing an opportunity to connect genotype and phenotype in extinct taxa. For example, reduced activity of MC1R has been shown to result in red hair in humans and cows and red or yellow hair in mice, horses, and dogs (4). Dark- and light-colored mammoth hair has been recovered from permafrost mummies, giving circumstantial evidence that mutations may occur in one of the genes involved in determination of hair color. We amplified the mammoth *Mclr* gene in 23 primary fragments varying in length from 101 to 138 bp. Three positions were found to be polymorphic in the individual sequenced, all resulting in amino acid changes [Thr²¹→Ala²¹ (Thr²¹Ala), Arg⁶⁷Cys, and

Arg³⁰¹Ser; positions relative to the elephant *Mclr* sequence] (Fig. 1A) (5). Because template damage may affect ancient DNA sequences (6), we sequenced each polymorphic position from at least three independent PCRs and, by using single-nucleotide polymorphism (SNP) typing, from additional specimens. The identification of homozygous individuals from these additional mammoth samples revealed most likely allelic constitutions of Thr²¹-Arg⁶⁷-Arg³⁰¹ (allele 1) and Ala²¹-Cys⁶⁷-Ser³⁰¹ (allele 2), respectively (5). Whereas Thr²¹ is highly variable among mammalian MC1R orthologs, substitutions of Arg⁶⁷ and Arg³⁰¹ (Fig. 1A) affect highly conserved residues. To investigate whether the two alleles show a functional difference, we constructed *Mclr* expression vectors that are identical to the inferred mammoth alleles on the amino acid level. The product of allele 1 shows high basal activity and robust responses to two agonists, α MSH (Fig. 1B) and NDP- α MSH (5), in a concentration-dependent manner. In contrast, the product of allele 2 shows an almost complete loss in basal activity and an ~65% reduction in efficacy (E_{max}) to both agonists. Functional testing of each position variable

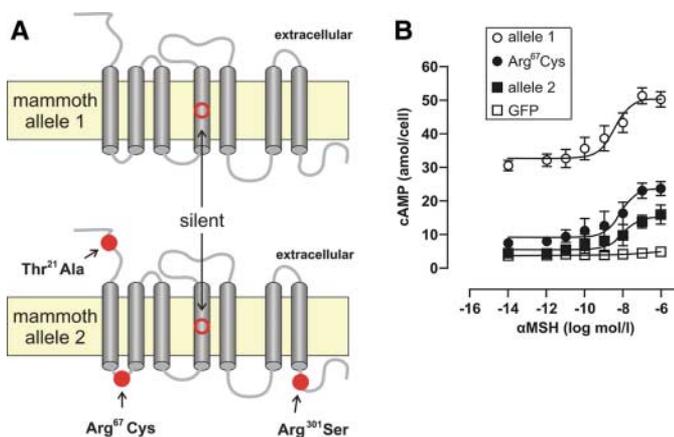


Fig. 1. (A) Localization of the fixed and polymorphic positions in mammoth *Mclr*. The seven transmembrane domain architecture of MC1R is depicted. Compared with the *Mclr* sequence of the African elephant, the approximate positions of the fixed silent position and polymorphic positions in the two mammoth alleles are circled. (B) Partial loss of function in mammoth MC1R variants. For functional characterization of the wild type (allele 1), Arg⁶⁷Cys and Thr²¹Ala-Arg⁶⁷Cys-Arg³⁰¹Ser (allele 2) constructs of the *Mclr* gene were transiently transfected into COS-7 cells and tested for agonist-induced cyclic adenosine monophosphate (cAMP) accumulation. Green fluorescent protein (GFP) plasmid-transfected cells served as controls. Intracellular cAMP was measured in response to increasing concentrations of α -MSH as described (5). Shown is the mean \pm SEM of four independent experiments, each performed in triplicate.

in the mammoth individually and all possible combinations of the three mammoth-specific amino acids revealed the Arg⁶⁷Cys substitution as the main cause for MC1R dysfunction (Fig. 1B) (5).

Pleistocene mammoths harbored both a fully and a partially active MC1R variant. Given an otherwise functional pathway for hair coloration, the reduction in activity of the Arg⁶⁷Cys variant would be sufficient to result in substantially lighter hair color. Intriguingly, light-colored populations of the beach mouse (*Peromyscus polionotus leucocephalus*) carry an *Mclr* allele that contains an Arg-to-Cys substitution at the homologous sequence position, and functional tests and crossing experiments have revealed both a reduction in basal and induced activity highly similar to that observed for the mammoth MC1R protein and a strong association between this amino acid polymorphism and adaptive coat color phenotype (7). These findings strongly argue for a phenotypic effect of this mutation also in the mammoth. Thus, it is possible that mammoth populations were polymorphic with regard to hair color, harboring both dark- and light-haired individuals. Although it is currently impossible to determine whether hair color was adaptive in mammoth, our study shows that functional analyses of nuclear genes are possible from late Pleistocene species. This opens up the possibility of studying a wide range of extinct species' features invisible in the fossil record.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5783/62/DC1
Materials and Methods
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References

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