Review of the Wooly Mammoth Genome

The Wooly Mammoth serves as a prime example when discussing the impact of climate change on biotic evolution. These large mammals lived from around 780,000 years ago to 4,000 years ago, during the Pleistocene and Holocene periods, and were one of the most abundant megafaunal species found, occupying a large area of land from Western Europe and Asia to North America. They are believed to have gone extinct in two main waves. The first was mainland extinction around 10,500 years ago, during the Pleistocene-Holocene transition, due to a variety of factors such a loss of habitat and human hunting (Palkopoulou et al., 2013). Changes in the climate such as rising sea levels led to the formation of small isolated populations on St. Paul Island and Wrangel Island, which went extinct in the second wave, during the mid-Holocene period (Palkopoulou et al., 2015). While the causes for extinction on St. Paul island are believed to be the lack of freshwater and shrinking land area, the reasons for extinction of the Wrangel island population are still unclear.

The paper, "Functional Architecture of Deleterious Genetic Variants in the Genome of a Wrangel Island Mammoth", by Erin Fry et al., studies the genetic makeup of the Wrangel island mammoth to try and see how it may have contributed to its extinction around 4000 years ago (Fry et al., 2020). Due to their geographic isolation, the size of this population reduced, leading to reduced genetic diversity and breeding among close relatives. This led to an accumulation of a high number of deleterious alleles in the Mammoth genome as compared to their ancestors on mainland Siberia, as well as present day Asian elephants. The paper identifies and characterizes these deleterious variants and shows that these might have affected development, reproduction and olfaction, thus playing a role in their eventual extinction.

Previously available genomes of 3 extant elephants and 3 wooly mammoths were used to identify homozygous nonsynonymous substitutions which were unique to these individuals. The three mammoths used in this study spanned from a period when mammoths were widespread(a 44,800 year old mammoth from mainland Oimyakon), to when they started declining(20,000

year old M4 mammoth) to the last known population(4300 year old Wrangel mammoth). Sequence alignment was done for all 6 genomes, using the African Savannah elephant(*L. africana*) as a reference, and around 106 to 594 unique nonsynonymous mutations were found across 106 to 583 genes in the 6 samples. These mutations were called unique only if they appeared in only 1 out of the 6 genomes. PolyPhen-2(Polymorphism Phenotyping v2), a software tool, was used to find the functional impact of each of these mutations, and classify them as 'benign' or 'probably damaging'. Finally, mouse knockout models and tissues where these genes were enriched were identified, and these were shown to have neurological and behavioral defects.

115 unique deleterious amino acid variants were identified in 112 genes of the Wrangel mammoth, out of which 4 variants were studied in detail. These included the HYLS1, NKD1, NEUROG2 and OR5A1. The regions of the gene where these mutations occurred were highly conserved throughout mammals, and hence mutations in those regions were thought to be potentially deleterious.

The Wrangel Island mammoth was found to have the P119L mutation (proline at 119th position replaced by leucine) in the HYLS1 gene which codes for the hydrolethalus syndrome protein 1, an important protein for ciliogenesis. To test the functional effect of this mutation, the HYLS1 gene was knocked out from *Xenopus* frogs, resulting in a phenotype which showed a serious defect in cilia assembly. The regular HYLS1 gene and the Wrangel Island mutant were added to this KO model, and it was observed that the regular gene rescued the KO model, while the mutant could not. Mutations in the HYLS1 gene are known to cause hydrolethalus syndrome, a lethal perinatal developmental disorder which causes brain malformation, as well as Joubert syndrome, which results in impaired balance and coordination. Therefore, it is possible that this

mutation had deleterious effects on the development of the Wrangel Island mammoths.

A luciferase reporter assay was conducted in elephant dermal fibroblasts to check the effect of the NKD1 A88V mutation (alanine replaced by valine). The protein produced by the NKD1 gene acts as a passive antagonist for the Wnt/TCR-LEF signaling pathway, and mutations in this leads to defects in sperm morphology. It was found that while the ancestor to the Wrangel Island mammoth (AncYakut) NKD1 gene reduced the expression of luciferase in the assay, meaning that it was a good antagonist, the mutated Island NKD1 gene did not affect luciferase expression. Thus, this mutation was a loss of function mutation, and could have led to reduced male fertility.

The third gene that was studied was the NEUROG3 gene, which is crucial for endocrine cell development. It was found through luciferase assays that the G195E substitution(glutamic acid replaces glycine) in the Island genome led to the overexpression of the gene when compared to the ancestral genome, meaning that this mutation was a hypermorphic one. This mutation could have had an effect on the insulin production of the Wrangel Island mammoth.

Previous studies showed that the Island mammoth genome showed a high rate of pseudogenization in olfactory receptors. This means that over time, the accumulation of mutations in these genes led to their loss of function or inactivation. One of these receptors with possibly deleterious amino acid substitutions in the Island mammoth genome was the OR5A1 receptor, in which the 193 amino acid (serine) was replaced by phenylalanine. The OR5A1 gene codes for a sensor which senses rose ketones and helps in the detection of herbaceous flowering plants. To test the effect of this mutation on the phenotype of the individual, an odorant receptor assay was done, using the ancestral non-mutated gene and the Island OR5A1 gene with the S193F substitution. It was found that the ancestral gene showed high sensitivity to rose ketones, while the Island gene did not, indicating that this was a loss of function mutation. Since herbaceous plants were a major component in the diet of mammoths, this mutation could have affected their ability to detect these plants. However, it could also simply mean that the floral composition on Wrangel island was different from the mainland, leading to a shift in their dietary preferences which was reflected in their genome.

It is known from previous studies that the geographic isolation of the Wrangel Island population led to a reduced population size. The estimated size of the Wrangel Island population was around 300 to 500 individuals, much below the minimum viable population size needed for preventing loss of genetic diversity and the accumulation of deleterious alleles. While this paper did not explicitly show how climate change was the cause of extinction of the Wrangel Island mammoths, it does show that some of the mutations found in the Island mammoths may have resulted in abnormal phenotypes and were deleterious. A small population of 300 individuals would be unable to effectively remove these harmful mutations, and thus over a long period of time, they would get accumulated in the population. This would lead to a 'mutational meltdown',

a positive feedback loop wherein accumulation of mutations causes reduced fitness and fertility of individuals, thus reducing the population size further and ultimately leading to their extinction.

Thus, we can infer that though the extinction of the Wrangel Island mammoth did involve a variety of factors, such as changes in vegetation and human activity, climate change did play a significant role in the extinction of the Wooly Mammoth. Global warming during the end of Pleistocene led to the shrinking of glaciers and subsequent rise of sea levels. This led to certain areas of land being separated from others, resulting in the geographic isolation of some animal species due to the formation of physical barriers. The small populations then experienced a 'mutational meltdown', and eventually, extinction. This paper used software and functional validation to show that Wrangel mammoths may have suffered adversely due to isolation and reduced population size. However, there were some limitations in this study, such as the inability

to do forward and reverse genetic experiments and the lack of a large sample size, and larger

samples are needed to come to any definitive conclusions.

References:

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