Current Biology

Complete Genomes Reveal Signatures of Demographic and Genetic Declines in the Woolly Mammoth

Highlights

- Complete high-quality genomes from two woolly mammoths were sequenced and analyzed

- 40,000-year time difference between samples enabled calibration of molecular clock

- Demographic inference identified two severe bottlenecks in the species' history

- One of the last surviving mammoths had low heterozygosity and signs of inbreeding

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In Brief

Palkopoulou et al. sequenced complete genomes from two woolly mammoths, including one dated to just before the species' extinction. Demographic inference indicates an ancient bottleneck and a decline at the start of the Holocene. Comparative analyses show that the woolly mammoth was subject to reduced genetic variation prior to its extinction.

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Complete Genomes Reveal Signatures of Demographic and Genetic Declines in the Woolly Mammoth

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SUMMARY

The processes leading up to species extinctions are typically characterized by prolonged declines in population size and geographic distribution, followed by a phase in which populations are very small and may be subject to intrinsic threats, including loss of genetic diversity and inbreeding [1]. However, whether such genetic factors have had an impact on species prior to their extinction is unclear [2, 3]; examining this would require a detailed reconstruction of a species' demographic history as well as changes in genome-wide diversity leading up to its extinction. Here, we present high-quality complete genome sequences from two woolly mammoths (*Mammuthus primigenius*). The first mammoth was sequenced at 17.1-fold coverage and dates to ~4,300 years before present, representing one of the last surviving individuals on Wrangel Island. The second mammoth, sequenced at 11.2-fold coverage, was obtained from an ~44,800-year-old specimen from the Late Pleistocene population in northeastern Siberia. The demographic trajectories inferred from the two genomes are qualitatively similar and reveal a population bottleneck during the Middle or Early Pleistocene, and a more recent severe decline in the ancestors of the Wrangel mammoth at the end of the last glaciation. A comparison of the two genomes shows that the Wrangel mammoth has a 20% reduction in heterozygosity as well as a 28-fold increase in the fraction of the genome that comprises runs of homozygosity. We conclude that the population on Wrangel Island, which was the last surviving woolly mammoth population, was subject to reduced genetic diversity shortly before it became extinct.

RESULTS AND DISCUSSION

The woolly mammoth is considered to have been one of the most abundant megafaunal species during the Middle to Late Pleistocene, yet along with ~70 other species of large mammals, it became extinct throughout most of its range at the Pleistocene/Holocene transition ~11,000 years ago [4]. A few small, isolated populations persisted into the Holocene on islands off the coasts of Siberia and Alaska that had become isolated from the mainland by rising sea levels [5, 6]. The last known population inhabited Wrangel Island until ~4,000 years ago [7].

We extracted DNA from the remains of ten woolly mammoths from Wrangel Island (Figure 1A) and used shotgun sequencing on a pool of indexed libraries to assess their levels of preservation (see Supplemental Experimental Procedures for full details). This allowed us to identify a molar tooth that contained a high proportion of mammoth DNA (~80%; Table S1). With a direct calibrated radiocarbon age of ~4,300 calendar years (Figure 1B; Table S1), this represents one of the most recently dated specimens that has been discovered on the island [7].

We also extracted DNA from a soft tissue sample obtained from a juvenile Siberian mammoth found in the Oimyakon District of Yakutia in northeastern Siberia (Figure 1A) and dated to ~44,800 calendar years ago [8] (Figure 1B). This individual, henceforth referred to as Oimyakon, was a member of the widespread woolly mammoth population that inhabited continental Eurasia during the Late Pleistocene.

We generated double-stranded DNA libraries from extracts of both specimens using uracil-DNA glycosylase treatment to excise uracils, following established protocols [9, 10]. As a reference, we used the genome of the African savanna elephant (*Loxodonta africana*) generated from a 6.8-fold coverage assembly at the Broad Institute (LoxAfr4). This assembly was based on paired-end long Sanger sequencing reads with a range of insert sizes, and optical mapping, allowing the construction of chromosome-length supercontigs. Approximately 76% of the sequences from the Wrangel individual aligned to the reference
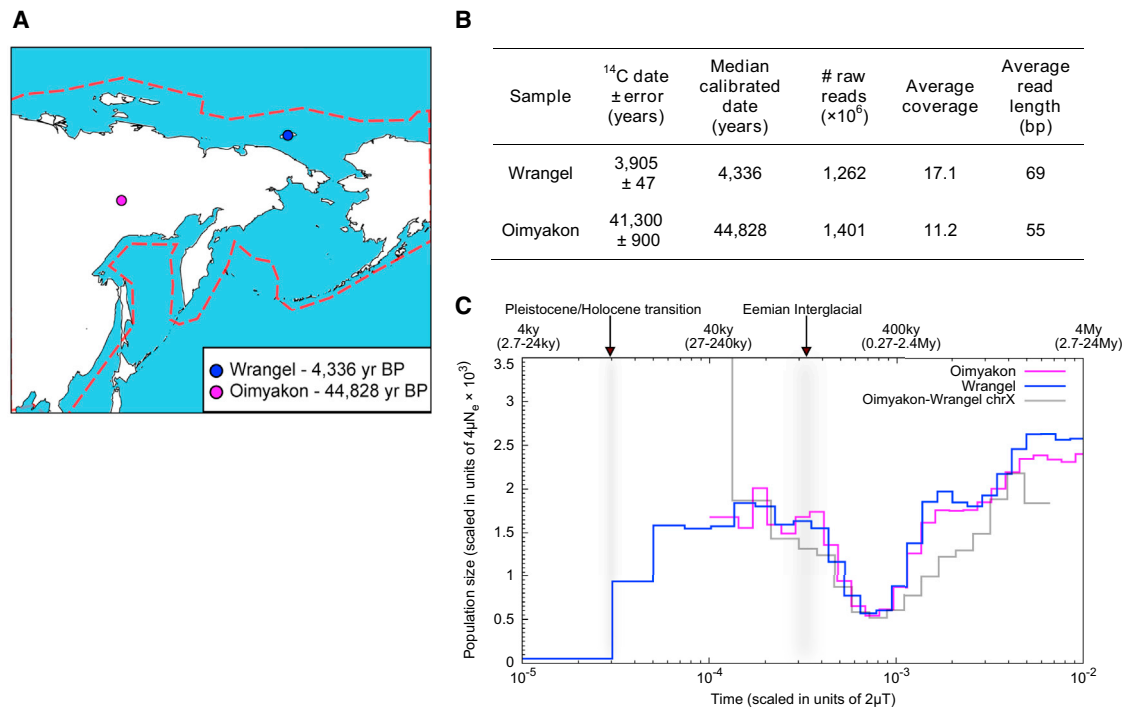
Figure 1. Geographic Location and Dating of Samples, Mapping Statistics of the Two Genomic Libraries, and Inference of Population Size Changes through Time

(A) Map indicating the sites where the mammoth samples were collected. The dashed red line indicates the approximate extent of the Beringia coastline during the last glacial maximum.

(B) Sample dating information and mapping statistics of the two libraries. ^{14}C date ± error refers to the radiocarbon age of each specimen and associated standard error. Median calibrated date refers to the median estimate of the calibrated radiocarbon date. Average read length (bp) refers to aligned sequences only.

(C) Population size history inferred using the PSMC method. Time is given in units of divergence per base pair on the lower x axis and in years before present on the upper x axis. The latter assumes the substitution rate estimated in this study based on the age difference between the two samples. (The range given in parentheses takes into account the uncertainty of the rate estimate as well the range of rate estimates obtained from paleontological calibration; see Table 1.) The PSMC curves of the Oimyakon genome and the pseudo-diploid chromosome X are empirically corrected for missing heterozygotes (false negatives = 30%) and are shifted along the x axis so that the former is aligned to the curve of the Wrangel genome and the latter ends at ~24,500 years ago, the average age of the two individuals (which was converted in units of divergence based on the mean substitution rate estimated in this study). The N_e of the PSMC curve of the pseudo-diploid chromosome X was scaled by 0.75. The Eemian interglacial period and the Pleistocene/Holocene transition are indicated by gray vertical bars assuming the mean substitution rate estimated in this study. See also Table S1 and Figure S1.

genome with an average length of 69 bp, yielding 17.1-fold average coverage. For the Oimyakon individual, 64% of sequences aligned and had average lengths of 55 bp, yielding 11.2-fold coverage (Figure 1B). We did not detect an excess of nucleotide misincorporations at the terminal positions of the sequences. Such misincorporations typically derive from cytosine deamination outside of CpG dinucleotides; their absence indicates that uracil excision was effective in both libraries (Figure S1). Phylogenetic analysis of the complete mitochondrial (mt) genomes from both individuals together with previously published woolly mammoth mitochondrial genomes [11] placed the Wrangel individual within mtDNA clade I [12] and the Oimyakon individual within mtDNA clade II [12] (Figure S2). These two mitochondrial clades have been proposed to represent two highly divergent populations or species [11, 13].

We inferred the history of population size changes in the ancestors of the two individuals using the pairwise sequentially Markovian coalescent (PSMC) method [14]. This approach uses the density of heterozygous sites across the diploid genome of

a single individual to infer the distribution of the time to the most recent common ancestor (TMRCA) between the two alleles across all chromosomes. This in turn can be used to infer effective population size (N_e) changes over time since effective population size is inversely proportional to coalescent rate. The estimated population size histories were qualitatively similar for Wrangel and Oimyakon but were offset by a fixed amount (Figure S3A), as might be expected since Oimyakon was around $\Delta T = 44,828 - 4,336 = 40,492$ years older than Wrangel. We inferred the number of missing substitutions per base pair in the Oimyakon genome that would be needed in order for the two populations to have the most concordant curves to be d = 0.0001 (range = 0.00004–0.00015) per base pair (Figure S3C). We then used this estimate to infer a substitution rate per base pair since we know that the rate of accumulation of divergent sites between samples is fully determined by two times the product of the substitution rate and time: $d = 2\mu(\Delta T)$ [15]. The estimated substitution rate is $\mu = 1.23 \times 10^{-9}$ (range = 0.49×10^{-9}–1.85×10^{-9}) per base pair per year, which is equivalent to 3.83×10^{-8}

Table 1. Dating of Different Events in Years and N_e Estimation Using Alternative Substitution Rate Calibrations

	Substitution Rate (μ)	Recent Bottleneck[a]	Earlier Bottleneck[b]	Wrangel-Oimyakon Split[c]	Wrangel-Oimyakon Split[d]	Wrangel Bottleneck N_e[e]
Mean (μ)[f]	1.23×10^{-9}	12,195	284,553	49,877	53,000–64,000	328
Upper limit (μ)[f]	1.85×10^{-9}	8,108	189,189	41,400	50,000–57,000	218
Lower limit (μ)[f]	0.49×10^{-9}	30,612	714,286	88,077	65,000–93,000	823
Upper limit (μ)[g]	0.6×10^{-9}	25,135	586,486	76,717	61,000–84,000	676
Lower limit (μ)[g]	0.21×10^{-9}	70,541	1,645,946	170,896	91,000–155,000	1,896

[a]Estimated time for the recent decline in N_e observed in the PSMC curve of the Wrangel genome.
[b]Estimated time for the earlier decline in N_e observed in the PSMC curves of both mammoth genomes.
[c]Split time between the Wrangel and Oimyakon individuals inferred from the PSMC analysis of the pseudo-diploid chromosome X.
[d]Split time between the Wrangel and Oimyakon individuals inferred from the F(A|B) analysis using autosomal data.
[e]N_e for the Wrangel population following the recent bottleneck inferred by the PSMC analysis.
[f]Mean, lower, and upper limit of the range of substitution rates (per bp per year) estimated in this study by lining up the two PSMC curves.
[g]Upper and lower limit of the substitution rates (per bp per year) obtained from paleontological calibration, assuming 6.2–17.4 million years for the genetic divergence time between African and Eurasian elephants (see Supplemental Experimental Procedures for details).

(range = 1.53×10^{-8}–5.73×10^{-8}) per base pair per generation assuming a generation time of 31 years [16]. Our inference that the substitution rate in mammoths is twice as high as the substitution rate recently estimated in humans using the same method ($\mu = 0.43 \times 10^{-9}$ per base pair per year) [15] may initially seem surprising since earlier studies have suggested that substitution rates in elephants may have been half that between humans and chimpanzees (an apparent factor of four difference) [13, 17]. However, there is substantial statistical uncertainty in all of these estimates, and part of the apparent discrepancy may have nothing to do with the accuracy of our rate estimate in mammoths and instead may reflect a major slowdown in the substitution rate in the hominin lineage [18]. In all of the analyses that follow, we use the PSMC-based point estimate of the substitution rate, which we view as the most accurate available, as this is the most direct estimate of the rate of substitutions that is available over the last tens of thousands of years of mammoth history, which are the main focus of this study. However, to be conservative, we also always quote a range of uncertainty of 0.21×10^{-9}–1.85×10^{-9} per base pair per year, which is a union of the range of statistical uncertainty around the point estimate (0.49×10^{-9}–1.85×10^{-9} per base pair per year) as well as an independent estimate of the substitution rate of 0.21×10^{-9}–0.6×10^{-9} per base pair per year that we obtained based on a more traditional calibration to the paleontological record. Specifically, we assume a genome-wide average genetic divergence time $T_{div(African-Eurasian)}$ = 6.2–17.4 million years for African and Eurasian elephants and note that the divergence per base pair between the African savanna elephant genome and Wrangel is $D_{African-Eurasian}$ = 0.0074 per base pair, which gives the substitution rate estimate range via the formula $\mu = D_{African-Eurasian}/2T_{div(African-Eurasian)}$ (see Supplemental Experimental Procedures for full details).

The Wrangel and Oimyakon individuals appear to have nearly identical demographic trajectories, once we line up the curves to account for the difference in the ages of the two samples (Figure 1C). In both genomes, we infer a dramatic reduction in N_e during the Middle or Early Pleistocene with a point estimate of 285,000 years ago (range of 189,000–1,646,000 years ago) (Table 1). A similar population bottleneck followed by population

expansion has previously been suggested based on mtDNA data [19]; however, the estimated time of this event was associated with the penultimate interglacial period (the Eemian [20], 116,000–130,000 years ago). The timing of the decline in N_e inferred using PSMC analyses of both genomes, across the entire range of dates obtained by our substitution rate estimates (Table 1), definitively predates the Eemian and thus does not seem to reflect this particular climatic event. Interestingly, ancient horses from Taymyr in Russia exhibit a reverse demographic pattern compared to woolly mammoths, with a demographic expansion at ~280,000 years ago and a decline during the Eemian as inferred using the PSMC analysis [21]. Following the population size recovery, N_e in woolly mammoths appears to have remained comparatively stable until a drastic reduction at ~12,000 years ago in the history of the Wrangel mammoth's genome (8,000–71,000 years; Table 1). Our best estimate for the timing of this steep decline in N_e coincides with the Pleistocene/Holocene transition and the subsequent isolation of Wrangel Island due to rising sea levels [7] and simultaneous disappearance of mammoths from mainland Eurasia [4]. We note that the upper limit of our range of uncertainty for this estimate (up to 71,000 years) is incompatible with the population history of the two woolly mammoths since the most recent decline, inferred in the Wrangel mammoth's genome, appears to have occurred after the death of the Oimyakon individual.

To test the hypothesis that woolly mammoths carrying clade I and II mtDNA haplotypes represented highly divergent populations, we estimated the divergence time of the ancestral populations of the Wrangel and Oimyakon individuals using two independent methods. First, the proportion of sequences mapping to chromosome X suggests that both individuals were males, which allowed us to construct a pseudo-diploid genome by combining their X chromosomes and estimate rates of coalescence between their ancestral populations [14]. The coalescence rate for the pseudo-diploid X chromosome is inferred to have changed over time in a similar manner as for the Wrangel and Oimyakon autosomes until the split of the two populations. The estimated split time dates to just before the death of the Oimyakon individual; after this period the PSMC estimates a sharp increase in N_e to an unmeasurably large size, reflecting
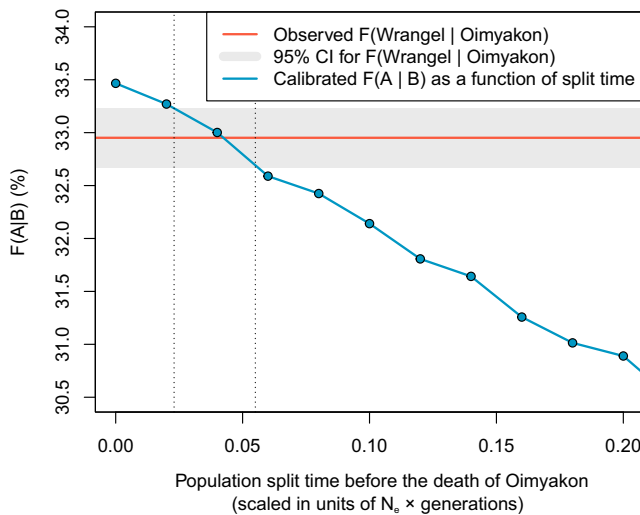
Figure 2. Population Split Time Estimate between the Wrangel and Oimyakon Populations

The probability F(A|B) of observing a derived allele in population A (Wrangel) at a heterozygous site in population B (Oimyakon) is obtained by simulating the history of population B (Oimyakon) as inferred using the PSMC method. Polarization of the alleles was based on the African savanna elephant, which was assumed to carry the ancestral state. The vertical dotted lines indicate the split times between the Wrangel and Oimyakon populations that encompass the confidence interval of the observed F(Wrangel|Oimyakon). Time on the x axis represents the population split time before the death of the Oimyakon individual and is scaled by N_e and generation time, where N_e indicates the terminal effective population size of the Oimyakon individual as inferred using the PSMC analysis. See also Figure S2.

an absence of detected coalescent events as would be expected if the populations were separated (Figures 1C and S3B). Similarly, the split time (T) between the populations represented by the Oimyakon and Wrangel genomes was estimated at ~50,000 years ago (range 41,000–171,000 years ago; Table 1), indicating that the Wrangel and Oimyakon populations shared ancestry until shortly before the death of the Oimyakon individual. To further investigate the split time of the ancestral populations, we used an independent approach for estimating population split times that uses the probability of a SNP across the diploid genome that is heterozygous in one population (Oimyakon) being derived in a second population (Wrangel) as a function of population split time [22, 23]. Based on this method, the Wrangel and Oimyakon populations were estimated to have split from each other 53,000–64,000 years ago (range 50,000–155,000 years ago) (Table 1; Figure 2). Overall, these results contrast sharply with those from a previous genomic study based on two low-coverage autosomal genomes. The previous study suggested coalescent times of ~1–2 million years between individuals that carried clade I and II mtDNA haplotypes and proposed that these individuals may have represented different species or highly divergent populations [13]. Based on the findings from the two autosomal genomes presented here, we conclude that there are multiple lines of strong evidence against this hypothesis. The observed discordance between nuclear and mtDNA estimates of divergence is not surprising if we consider the biology and natural history of the Elephantidae.

Females are largely non-dispersing, and this tends to produce deeper coalescent times for mtDNA lineages compared to nuclear coalescent dates [24]. Conflicting genetic patterns between nuclear and mtDNA have been observed in modern elephants [25]. Indeed, all extant elephant species exhibit mtDNA coalescent dates that are about as old as the divergence date between mtDNA clades I and II in woolly mammoths [26].

Average autosomal heterozygosity in the Wrangel individual was 1.00 heterozygous site per 1,000 bp (confidence interval [CI] 0.99–1.02), which is 20% lower than the heterozygosity observed in the Oimyakon individual (CI 1.23–1.27 per 1,000 bp; Table S2). This is consistent with results from previous genetic studies [27, 28] indicating a loss of genetic diversity as mammoths became isolated on Wrangel Island and is likely a consequence of a reduced N_e due to the island's small size and estimated low carrying capacity [27]. When compared to genome-wide diversity levels in extant organisms (Figure 3A), we find that heterozygosity in the Wrangel individual was low, but not exceptionally so. Diversity in the Wrangel genome falls close to that observed in humans, bonobos, eastern lowland gorillas, and western chimpanzees, which have also experienced dramatic declines in population size during their history as inferred from PSMC analyses [29]. On the other hand, the Wrangel individual harbored higher genomic diversity than that observed in several other endangered taxa such as lions, tigers, Tasmanian devils, snow leopards [30], and polar bears [31], most of which have experienced recent declines in population size. We speculate that the particularly low heterozygosity in these latter taxa may be partly due to these species all being predators, which typically occur at low population densities and thus have had comparatively small effective population sizes throughout their history.

To examine the genomes for runs of homozygosity (ROHs), we estimated the inferred TMRCA at every position across the genome of each individual using the PSMC method. An extreme excess of ROHs was detected in the Wrangel genome (23.3% of the genome constituted ROHs) compared to the Oimyakon genome (0.83% of the genome constituted ROHs), providing evidence of a small N_e in the recent history of the Wrangel individual (Figures 3B and 3C), consistent with the PSMC results. Most of these regions of low heterozygosity were found to span a few million base pairs and were distributed across all autosomes (Figure S4). ROHs of such length typically occur from background relatedness associated with limited population size in the last dozens of generations rather than due to recent mating of closely related individuals, which would be expected to produce much longer stretches [32]. Thus, we find no evidence of inbreeding in the sense that the parents of the Wrangel individual were particularly close relatives. Instead, it seems likely that the large proportion of ROHs in the Wrangel genome is due to a cumulative effect of recurrent breeding among distant relatives, which is consistent with a small Holocene effective population size on Wrangel Island. Further genomic analyses on additional specimens from Wrangel [7] should be able to resolve this issue and enable detailed reconstruction of population size changes over the Holocene period when Wrangel Island became separated from the mainland. Additional analyses on mammoths representing the last remaining mainland populations in Eurasia and North America would also be valuable in order to shed light
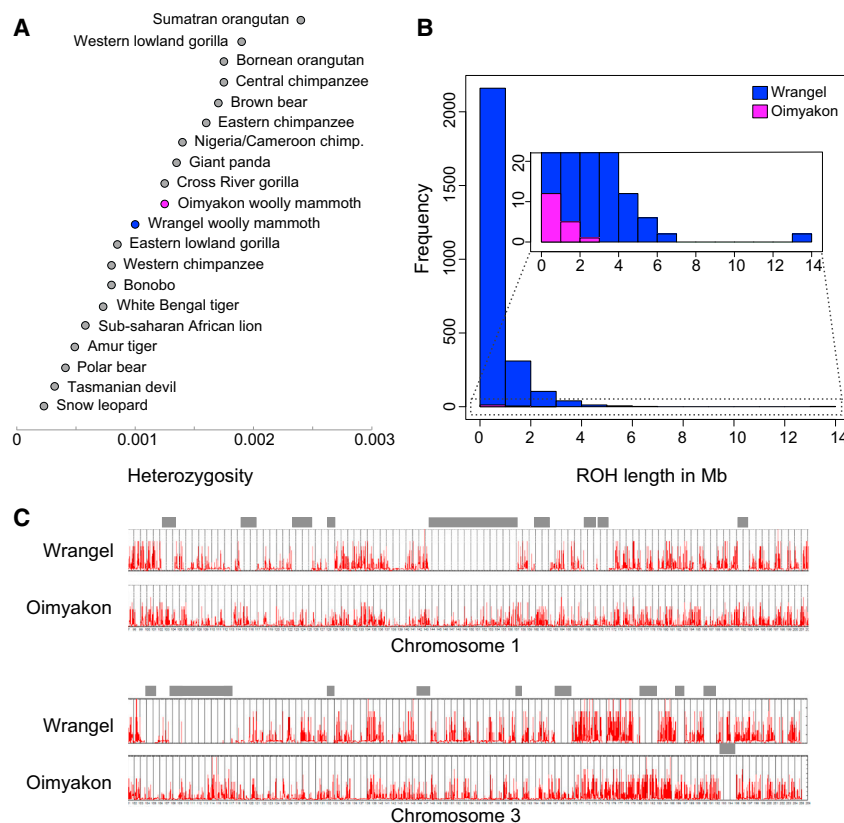
Figure 3. Estimates of Genome-Wide Heterozygosity and Runs of Homozygosity

(A) Comparison of genome-wide heterozygosity estimates in different taxa/populations [29–31] and the two woolly mammoths. See also Table S2.

(B) Histogram of the lengths (in Mb) of runs of homozygosity (ROHs) longer than 0.5 Mb within the Wrangel (blue) and Oimyakon (purple) genomes. The inset shows a magnification with a different y axis scale.

(C) Inferred time to the most recent common ancestor (TMRCA) for the two alleles of a single individual along 106 Mb of chromosome 1 and chromosome 3, with Wrangel on top and Oimyakon at the bottom. The y axis shows the TMRCA, and the x axis shows positions along the chromosome. Gray bars indicate regions longer than 1 Mb that are inferred by the PSMC method to have coalesced recently and represent ROHs. See also Figure S4.

seems plausible that the low genetic variation detected in this study may have had a negative impact on the fitness of the Wrangel Island population and thus may have contributed to its subsequent extinction. The results presented here also highlight the value of sequencing ancient genomes from specimens that predate population declines to establish baseline levels of genome-wide diversity. In conservation biology, this approach can be used to directly quantify the amount of diversity lost in threatened species.

on whether these populations were also subject to reduced genetic variation prior to their extinction at the end of the last glaciation.

Interestingly, the lower heterozygosity in the Wrangel genome can largely be explained by its higher proportion of ROHs. This implies that heterozygosity is similar between regions of the Wrangel genome that are not in ROHs and the Oimyakon genome as a whole, which may seem odd given the high likelihood of genetic drift in the Holocene Wrangel population. We suggest two possible hypotheses that might explain this observation. First, it is possible that positions in the Wrangel genome that are not in ROHs are places where the TMRCA between the Wrangel individual's chromosomes extends all the way back to the time when Wrangel was a part of the mainland (i.e., Beringia). If this is the case, then non-ROH regions should reflect the genetic diversity that existed at the time before N_e declined (as a consequence of the isolation on Wrangel). Alternatively, the observed similarity in diversity may be an effect of the Wrangel genome being of higher quality and/or coverage than the Oimyakon genome, which could have allowed more heterozygous positions to be called in the analyses (due to lower false-negative rates). If this is the explanation, the estimated 20% reduction in the Wrangel genome's diversity may be an underestimate.

In conclusion, our finding of an overall reduced genome-wide diversity in one of the last surviving mammoths constitutes the first direct observation of genetic stochasticity in a species shortly before its extinction. Given that small population sizes in wild animals often lead to reduced individual fitness [33], it

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