therapeutic targets for degenerative diseases associated with extracellular protein aggregates.

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Evolution: Untangling the Woolly Rhino's Extinction

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The woolly rhinoceros was a charismatic inhabitant of the frigid steppes of Pleistocene Eurasia. Now, the genome of an 18,500-year-old woolly rhino has been sequenced. It points to a thriving population less than 5000 years before the species disappeared.

Humans marveled at the Late Pleistocene woolly rhinoceros (*Coelodonta antiquitatis*), as testified by cave art from the period (Figure 1); but did our ancestors kill off the species? Around the end of the last ice age, Northern Eurasia, home of the woolly rhinoceros, lost over a third of its megafauna — animal species weighing over 44 kg [1]. To what degree humans or climate change, or a mix of both, caused the extinction of Late Pleistocene megafauna has been much debated. A '*Blitzkrieg*' hypothesis posits that human hunting wiped them out [2]. In North America, megafaunal extinctions occurred within a period of less than 1000 years, around the time that humans arrived [3]. By contrast, extensive fossil radiocarbon dating has shown that last occurrence dates for large animals across Eurasia are staggered across tens of thousands of years [1]. Warmer climates and loss of open habitats are documented near the time the woolly rhino disappears from the fossil record, suggesting a role for climatic factors in its demise [4]. Yet, humans were also present across most of its range at that time. A new study by Edana Lord, Love Dalén and colleagues [5] in this issue of *Current Biology* has generated the first nuclear genome sequence of a woolly rhinoceros [5]. This rhino lived around 18,500 years ago in northeast Siberia, many thousands of years after humans entered much of the woolly rhino range, and a few thousand years before the species' extinction around 14,000 years ago [1]. What insights might a single woolly individual possibly provide into the rhino's extinction? As it turns out, quite a few.

Distant Kin

The rhino genome sequenced by Lord and colleagues [5] indicates that the individual belonged to a genetically 'healthy' population. The level of inbreeding was modest, indicating that the population was large enough for its parents to have a low level of kinship [5]. If the rhino's parents had been relatives, they would share regions of their genome that both inherited from the same ancestral chromosome. Inbred progeny can then inherit two copies of the same ancestral chromosome segment, one from each parent. Where maternal and

Figure 1. A woolly rhino painted around 30 thousand years ago in Chauvet Cave.
Were ancient humans who encountered the woolly rhinoceros responsible for its demise? (Photo: inocybe.)

paternal chromosomes are identical in the inbred progeny, the genome will show runs of homozygosity (ROH), genomic deserts lacking allelic variation. The more closely related the parents, the greater the number of ROH, and the longer the ROH segments. The sequenced woolly rhino did show ROH, comprising 5.9% of its genome. However, these ROH were of short length, suggesting that the rhino's parents were not very close kin. One would infer a population that was large enough for outbreeding, but with gene flow from outsiders low enough to permit the population to consist of distant kin.

Additional support for a relatively large population is provided by the level of heterozygosity detected in the woolly rhino genome [5]. A large population can maintain substantial genetic diversity. Squeeze the population down to a small size and diversity is lost. Heterozygosity in the woolly rhino genome was much higher than in the genome of a mammoth isolated in a small island population, and was also higher than in the genome of an outbred mainland woolly mammoth, or compared to living rhino species [5,6]. The woolly rhino population remained large enough to maintain considerable genetic diversity [5,6].

Even a single genome is sufficient for ancestral population sizes to be inferred, using an analysis called 'pairwise sequentially Markovian coalescent' (PSMC) [7]. Genomic regions of high diversity are remnants of a time when the rhino population was large, while regions of low diversity are from times when population size was low. PSMC determines the lengths of these regions of varying diversity. Short regions of the genome are more ancient than longer regions. That's because during meiosis, maternal and paternal chromosomes recombine to form mosaic chromosome combinations. More generations give more opportunities for these chromosomal rearrangements to shorten the sizes of genomic fragments travelling down a pedigree. Longer fragments in the chromosomal mosaic indicate less recombination, thus more recent events. PSMC scans the genomic mosaic for fragments of varying diversity (reflecting population size) and determines the lengths of the fragments (reflecting the number of generations). And voilà — effective population size for the woolly rhino can be estimated back across tens and hundreds of thousands of years using a single assembled genome [7]. The 'effective population size' is typically smaller than the actual census size, because in real populations genetic diversity is lost at a faster pace than under simplifying assumptions made by PSMC. Importantly, PSMC analysis found that the woolly rhino effective population size increased some 30 thousand years ago, with the larger size maintained until the lifetime of the sequenced individual.

Woolly rhino populations were geographically subdivided in the distant past, as Lord and colleagues [5] inferred by sequencing mitochondrial genomes from 14 Late Pleistocene specimens (one of which was rhino tissue found inside the stomach of a puppy frozen 14,000 years ago). A mitogenome is distinct from a nuclear genome, and represents a single evolutionary trajectory as there is no recombination. In a phylogenetic tree, the woolly rhino mitogenomes fell into two distinctive groups or clades that had split about 205,000 years ago [5]. This very ancient split may have occurred when the geographic range of woolly rhinos contracted into small isolated refugia during one of the major warm periods (interglacials) that separate the various Pleistocene ice ages. By the Late Pleistocene, their geographic separation had ended, as both clades are found across northeast Siberian populations until the very end [5]. Yet, within the last ice age, there were minor warm periods (interstadials) during which rhino range may have contracted. Then during colder periods (stadials) the populations may have again merged geographically. Populations drift apart when genetically separated, and reuniting them causes an increase in genetic diversity. This is interpreted by PSMC as a higher effective population size and may account for part of the increase around 30 thousand years ago detected by PSMC. However, there was probably also an increase in the actual number of rhinos at that time.

Winter Wonderland

More arid and glacial conditions set in 30 thousand years ago, leading to range expansion for the ice age steppe vegetation favored by woolly rhinos, which likely increased the rhino population size [4,8]. One can examine the mitogenome tree not only for ancient subdivisions, but for distortions reflecting changes in population size. Like the nuclear genome, mitochondrial DNA

patterns suggest that the woolly rhino population was large and stable or expanding near the end of the Pleistocene [4,5]. Ancient DNA studies have detected similar signatures of population increase around 30 thousand years ago for other species that relied on steppe vegetation, including Eurasian horse, reindeer and musk ox [4]. The woolly rhino effective population size is inferred to be higher using mitochondrial than using nuclear DNA [5]. Because mitogenomes are only passed on by females, this outcome could result from male–male competition [9] — Paleolithic art shows what look like sparring woolly rhino bulls. If the number of sires was limited, this would explain the runs of homozygosity scattered across the genome, vestiges of the chromosomes of highly successful male individuals [5].

Several ancient species within the woolly rhino genus *Coelodonta* have been described. The genus shows morphological changes in posture, skull and teeth as the rhinos progressively adapted from being mixed feeders to being highly specialized grazers in tundra-steppe environments [10]. Adaptations for survival in dry and in cold conditions may originally have evolved in response to high elevation, since a very ancient rhino species has been found in Tibet [10]. Lord and colleagues [5] examined the woolly rhino genome for genes that might have evolved in response to cold climate. Strong candidates are the genes *TRPA1* and *KCNK17*, which are involved in sensing cold or heat [11] and display significant mutations in both the woolly rhino and its 'fellow traveler' the woolly mammoth [5,12].

For much of the Late Pleistocene, the woolly rhino happily grazed in the cold steppe across northern Eurasia, south into northern China, and west across most of Europe. But the rhino was ill-suited for the Bølling-Allerød interstadial beginning around 14,700 thousand years ago, which brought the warmest climate in 50,000 years. Open habitats were largely replaced by shrubs and trees, first in western, then in eastern Eurasia [1,8]. A survey involving over 200 reliably dated woolly rhino specimens reported the last fossil occurrences around 17 thousand years ago in Western Europe, around 15 thousand years ago in Eastern Europe (European Russia), with the last survivors either in the Urals or north-east Siberia around 14 thousand years ago [1,13,14]. The large effective population size inferred from the nuclear and mitochondrial genomes suggests that the rhino population was doing well in northeast Siberia 18,500 years ago [5]. Numbers were not collapsing then, even though humans had reached northeast Siberia many thousands of years earlier [15]. Perhaps the woolly rhino was done in by subsequent climate and habitat changes [4].

And yet, one wonders. Currently, humans threaten 32,000 species with extinction, including the five surviving rhinoceros species (https://www.iucnredlist.org). The woolly rhino's closest living relative, the Sumatran rhinoceros [16], has been persecuted for thousands of years, with at most 90 animals surviving in the wild [17,18]. Many large and slow-breeding animal species have become extinct in the last 50 thousand years, and this extinction is said to be unprecedented in the previous 55 million years, implicating the direct and indirect impact of humans [19]. Some Late Pleistocene archeofaunal sites in Europe and Siberia contain woolly rhino remains, including hunting tools made from rhino horn [20], with remains found at some Siberian archaeological sites less than 20,000 years old [4]. This is not proof of hunting by humans, and the rhino genome shows no signs of an ongoing population collapse at that time. It is possible that only habitat changes caused the ultimate demise of the woolly rhinoceros and that humans were not involved. Yet, one may be forgiven for making this statement with a skeptical eyebrow raised over a jaundiced eye.

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Membrane Biology: Disentangling Cellular Lipid Connections

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Biological membranes consist of a surprisingly high number of different lipid species. Little is known about how individual lipids cooperate in modulating cellular functions. A new study suggests an intricate interplay of sphingolipids with ether lipids in vesicular transport.

Membrane lipid species differ between the kingdoms of life, with their diversity increasing with organismal complexity. Our understanding of the fine-tuning of cellular functions resulting from the interplay of hundreds of different membrane lipid species is still very limited [1]. In mammals, membrane lipids are mainly composed of cholesterol, glycerophospholipids and sphingolipids. The huge diversity of lipid species found in membranes is made possible by the high variability of the building blocks that are used to make these lipids. Glycerophospholipids, for example, all share a glycerol phosphate backbone to which different types of head groups and hydrocarbon chains are linked. The hydrocarbon chains that differ in the length, number and position of double bonds are derived from either fatty acids (acyls) or alcohols (ether lipids and plasmalogens). The multiple ways in which these individual building blocks can be combined mean that cells are armed with an impressively large reservoir of different lipids that they can use on demand to generate a broad range of membrane lipid compositions. Also, different types of lipid species have different physico-chemical properties; upregulation or downregulation of specific lipid species therefore helps cells to respond to environmental changes. How changes in the amount of a specific lipid species impact other lipids, protein functions and overall membrane properties is still poorly understood, as are the stimuli that trigger membrane lipid plasticity. Although the pathways interlinking glycerophospholipids and sphingolipids are well described, exploring diversities in lipid functions at a molecular level is a challenging task. Techniques that specifically and acutely manipulate individual lipid species are emerging but are still scarce [2], and the interpretation of data is complicated by the fact that lipid alterations affect cells at many different levels.

In this issue of *Current Biology*, Jiménez-Rojo *et al.* [3] present an elegant study that sheds light on the co-dependency of different lipid species and their interplay in modulating intracellular transport. These authors performed a high-throughput CRISPR interference (CRISPRi) screen in mammalian K562 leukemic cells [4] that had been subjected to sphingolipid deprivation, in order to identify proteins and metabolic pathways that either provide resilience against the homeostatic imbalance or lead to hypersensitivity. To this end, a CRISPRi library was used to repress the expression of 16,000 genes. Cells were then treated with myriocin, an inhibitor of serine palmitoyltransferase, which is the first enzyme in *de novo* sphingolipid synthesis. Cells respond to myriocin with growth arrest, which was used as a readout to identify genes associated with either hypersensitivity or hyposensitivity to the treatment. In total, the authors detected approximately 180 gene products linked to hypersensitivity and 1.5-fold more hyposensitivity hits. Bioinformatic analysis of these genes showed that a surprisingly limited set of functional categories were affected. The most abundant functional group within the hypersensitivity hits was a subset involved in intracellular trafficking and cholesterol metabolism, demonstrating the intimate connection between lipid homeostasis and vesicular transport [5].