

scientists approach their research. Previous global-scale reconstructions of the past 24,000 years either focused on narrow time intervals to develop a full spatial picture of temperature changes<sup>13</sup>, or studied changes in temperature averaged over the globe to determine the evolution of the climate<sup>4,6</sup>. Osman *et al.* have effectively combined these approaches to produce a complete reconstruction of climate change through space and time.

There are, of course, limitations to the authors' work. No terrestrial data were included in the data set compiled, and few data are available for the central Pacific, Indian and Southern oceans, leaving some questions as to how accurate the reconstruction is across these large expanses of water and continents. This is particularly important when considering the warming that the study reports for the current interglacial epoch, because a reconstruction published last year using several hundred terrestrial climate records instead shows a cooling trend<sup>11</sup>, albeit without the help of model simulations. Osman *et al.* also relied on a single climate model, which might bias their results, because different models can produce different spatial patterns of climate change.

Drawing on the innovative data compilation and modelling methods of Osman and colleagues as a foundation, future work should focus on adding more terrestrial records to similar global temperature reconstructions. There is also a need for more model simulations that include water isotopes, so that the authors' approach can be repeated with different climate models. This will help researchers to better assess the degree of uncertainty associated with the temperature reconstruction.

Nonetheless, the work by Osman *et al.* is a triumph, and sets a new standard for the development of large-scale temperature reconstructions of the geological past. It should inspire climate scientists to undertake similar analyses, and perhaps even to consider temperature changes that occurred before the Last Glacial Maximum – fulfilling Kutzbach's vision of combining models and data to fully decipher the climate of the palaeo world.

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## Evolution

# Rhinoceros genomes uncover family secrets

Desire Lee Dalton & Stefan Prost

Genomes from living and extinct rhinos reveal that different species evolved as a result of geographic isolation. A comparison of DNA from different species also shows that rhinos have long displayed low genetic variability.

Historically, rhinos were once abundant throughout Europe, Asia and Africa<sup>1</sup>. Today, five species of rhinoceros survive as small populations in Asia and Africa, and are all threatened with extinction<sup>2</sup>. Although well studied, there is debate in the literature about evolutionary relationships between modern and extinct rhinos, with three hypotheses being proposed (Fig. 1a–c). Writing in *Cell*, Liu *et al.*<sup>3</sup> analyse contemporary and ancient rhinoceros DNA to piece together the puzzle of the rhino's evolutionary history.

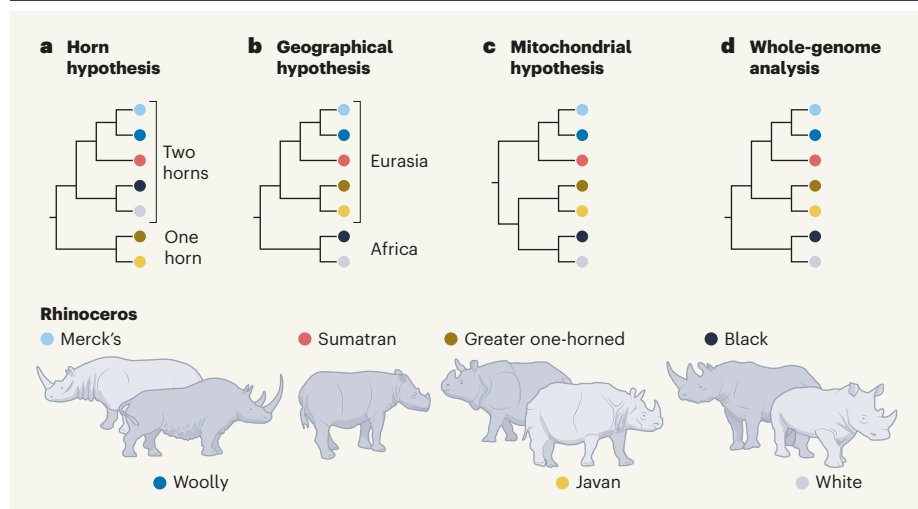
The authors analysed the genomes of five living rhinoceros species – namely black (*Diceros bicornis*), white (*Ceratotherium simum*), Sumatran (*Dicerorhinus sumatrensis*), greater one-horned (*Rhinoceros unicornis*) and Javan rhinoceros (*R. sondaicus*). They also analysed ancient DNA from the Late Pleistocene (the period of time ranging from about 126,000 to 11,700 years ago) obtained from bone and tooth samples of three extinct rhinoceros species: the woolly rhinoceros (*Coelodonta antiquitatis*), the Siberian unicorn (*Elasmotherium sibiricum*) and Merck's rhinoceros (*Stephanorhinus kirchbergensis*). The genomic data revealed that the rhinoceros lineage split approximately 36 million years ago, with the Siberian unicorn separating from a group that included all the other rhino species examined in the study.

The genomic analysis suggests that, approximately 16 million years ago, in the Miocene (which lasted from about 23 million to 5 million years ago), this group then split into two groups: African and Asian. This might have happened when the ancestor of living black and white rhinos moved from Eurasia into the African continent either because of changes in

climate or when a land bridge formed between the two land masses approximately 20 million years ago<sup>4</sup>. According to Liu and colleagues' analysis, the Asian group, located in Eurasia, further split into two groups approximately 14.8 million years ago (Fig. 1d). One group consists of the greater one-horned rhinoceros and the Javan rhinoceros (which is found only in a small part of the island of Java in Indonesia); the other includes the Sumatran rhinoceros (living on the neighbouring island of Sumatra), Merck's rhinoceros and the woolly rhinoceros – all of which have current or past geographical ranges that include parts of Asia.

However, Liu *et al.* obtained conflicting signals regarding the position of the different species in the family tree. Depending on the chromosomal region they analysed, the branching of the tree was similar either to that suggested by analysis of DNA in a cellular organelle called the mitochondrion (Fig. 1c) or to that based on geographical distribution (Fig. 1b). The authors attributed the inconsistency between analyses of different chromosomal regions to incomplete lineage sorting, whereby different rhinoceros species might have retained ancestral forms of genes and maintained gene flow between each other after these species formed. The occurrence of these phenomena can lead to the generation of trees that do not show actual relationships between species.

Following extensive analysis that took into account incomplete lineage sorting and gene flow, the authors report that the current genomic data set indicates that rhinoceros species evolved through geographical isolation (Fig. 1b), as previously proposed<sup>5</sup>. However, the authors noted that, to fully



**Figure 1 | Different hypotheses of the evolutionary history of rhinos.** **a**, The horn hypothesis<sup>8</sup> groups rhinoceros species on the basis of the number of horns (one or two). This hypothesis is supported by the shape (morphological) characteristics of cranial, dental or other skeletal remains, analysis of the proteins in ancient dental enamel<sup>9</sup> and the analysis of regions of the nuclear and mitochondrial genome<sup>10</sup>. **b**, The geographical hypothesis<sup>5</sup> groups rhinoceros species on the basis of their former or present geographical distribution. This hypothesis is supported by analyses of skull, skeleton and dental features<sup>11</sup> and the geographical distribution of current and historical rhinos, which were classified on the basis of examination of a limited number of genetic regions<sup>5</sup> and the protein collagen<sup>12</sup>. **c**, The mitochondrial hypothesis was proposed following analysis of the entire mitochondrial genome from different rhino species<sup>13</sup>. **d**, Liu *et al.*<sup>3</sup> examined whole genomes of modern and extinct rhinos and found that the proposed evolutionary history supported by the results of this analysis is consistent with the geographical hypothesis.

understand the evolutionary history of the rhinoceros family, certain limitations would need to be addressed. Only a small number of samples from extinct rhinoceros species could be analysed. In addition, analysis of ancient DNA is fraught with problems, because the DNA is often damaged and broken into short pieces, and genomes from other species have to be used to determine the correct order of the pieces of sequenced DNA.

Modern rhinoceros populations are reported to have low genetic diversity and high levels of inbreeding as their populations dwindle, making them vulnerable to future environmental change<sup>6</sup>. To further investigate genetic diversity, the authors looked at the number of variable sites throughout the genome (dubbed genome-wide heterozygosity) of living rhinos (black, white, greater one-horned and Sumatran) compared with that in samples of historic but still extant (Javan) and extinct rhinos.

To compare heterozygosity estimates, the authors focused on transversion mutations in which a purine nucleotide base (adenine or guanine) is replaced by a pyrimidine base (cytosine or thymine), or vice versa. They did not examine transition mutations, in which one pyrimidine or purine replaces another pyrimidine or purine, respectively, because such changes could potentially reflect ancient DNA damage. The authors further compared these heterozygosity values with those reported for other mammals. To do so, they recalibrated the transversion-based estimates

using known ratios of transitions to transversions. The authors also investigated levels of inbreeding by looking at the number of continuous regions in the genome that lacked variability.

These analyses revealed that living rhinoceroses show markedly lower diversity and higher levels of inbreeding than did the historical rhinos and extinct species. However, all the rhinoceros species included in this study had

### “Living rhinoceroses show markedly lower diversity and higher levels of inbreeding than did historical rhinos.”

lower genetic diversity in general compared with all other mammals tested, except for those in the family Felidae (cats).

Another important factor for the survival of threatened populations, besides genetic diversity, is mutational load. This refers to the number of deleterious mutations in the genome, and is a common approximation of fitness at the individual and population levels. The authors show that all studied rhinoceros species have a mutational load comparable to that of other present-day mammals. Liu *et al.* attribute the similarity of these mutational loads to the fact that the effective population sizes (the numbers of individuals that give

rise to the next generation) have long been low for all the rhinoceros species examined. Indeed, genomic analyses of other species targeted by conservation efforts, such as mountain gorillas<sup>7</sup>, have attributed low values for mutational load to long periods of low effective population sizes and high levels of inbreeding, which allowed effective purging of deleterious mutations. This has also been shown in the Sumatran rhinoceros: populations with high rates of inbreeding showed lower mutational load than did populations with less inbreeding<sup>6</sup>.

So, what does Liu and colleagues' study mean for future rhinoceros conservation? Rhinos worldwide face several threats, including poaching for illegal trade, and habitat fragmentation and loss. The authors determined that modern rhinos are genetically less diverse than ancient species and that the decline in genetic diversity is most probably due to human activities. However, low genetic diversity seems to be a long-term feature of the rhinoceros; thus, focused conservation efforts might be more fruitful than previously envisaged. The availability of rhinoceros genomes means that accurate and cost-effective genetic tests can be designed to monitor variability in isolated small populations. Furthermore, genetic tests could provide recommendations in terms of mixing of populations and selection of specific individuals to breed, to maximize diversity in future generations.

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