

## Opinion

## The Promise of Paleogenomics Beyond Our Own Species

Katherine Brunson<sup>1,2,\*</sup> and David Reich<sup>3,4,5</sup>

**Paleogenomics, also known as genome-wide ancient DNA analysis, is transforming our understanding of the human past, but has been much less intensively used to understand the history of other species. However, paleogenomic studies of non-human animals and plants have the potential to address an equally rich range of evolutionary, paleoecological, paleoenvironmental, and archaeological research questions. Three recent case studies of cave bears, horses, and maize provide examples of the ways that paleogenomics can be used to examine potential causes of extinctions and dynamic processes of domestication. Much more research in these areas is needed, and we conclude by highlighting key future directions.**

### Paleogenomic Research Is Underdeveloped Outside of Our Own Species

**Paleogenomic** (see [Glossary](#)) or genome-wide **ancient DNA (aDNA)** studies have transformed our understanding of the human past, but their impact on our understanding of the history of non-human animals and plants is far more limited. High-throughput sequencing methods have made it possible to regularly obtain data from hundreds of thousands of variable positions in the genome [single nucleotide polymorphisms (SNPs)] and even entire genomes from increasingly diverse sources drawn from archaeological, paleontological, and archival materials ([Box 1](#)). It is now possible in principle to learn a great deal about the past few hundred thousand years of history of extant species as well as the history of millions of recently extinct plants and animals that once lived on the planet. The sheer volume of remains from non-human species also offers far more raw material in the form of ancient bones, shells, feathers, hair, husks, and seeds than human remains ever will. The range and variability of ancient non-human organisms provides power to address evolutionary, ecological, social, and environmental questions in deep time, many of which cannot be well-addressed by aDNA studies of humans alone.

[Figure 1](#) highlights the underdevelopment of paleogenomic studies and **archaeogenomic** studies (i.e., paleogenomic studies based on remains drawn from human archaeological sites) outside our own species. At the time of writing in mid-December 2018, paleogenomic data from 2196 ancient individuals have been published at reasonably good quality; 87% of these were from humans ( $n = 1909$ ), 9.4% from animals ( $n = 207$ ), and only 3.6% from plants ( $n = 80$ ). The majority of these plant and animal samples either come from the temperate regions of the northern hemisphere (mostly Eurasia), or arctic regions where aDNA preservation is particularly favorable. Most research has focused on a limited number of taxa (most of the plant genomes shown in [Figure 1](#) are from maize). For the aDNA revolution to reach its full potential, however, it needs to expand its horizons to a wider variety of organisms, both living and extinct, and needs to get to the point where the great majority of genomes that are published are from species other than our own. Ancient genomes from a wider variety of species will also provide opportunities to improve laboratory methods, develop new statistical analyses, and develop a broader understanding of evolution.

### Highlights

To date, genome-wide analyses of ancient organisms have primarily focused on humans, despite the fact that there are millions of living and extinct plant and animal species that also can be studied with these techniques.

Natural history museums, archives, and archaeological collections harbor abundant sources of ancient DNA for studying past plant and animal genetic diversity around the world.

Ancient DNA provides access to genomic data covering hundreds of thousands of years, allowing for the investigation of evolutionary, ecological, social, and environmental questions in deep time, especially regarding the ways that humans have interacted with other species and modified past ecosystems and environments.

Case studies of cave bears, horses, and maize highlight the power of paleogenomic data to shed light on extinctions, admixture between domestic and wild animals, and the gradual selection for domestication genes during plant and animal domestication.

It is time to apply the power of genome-wide ancient DNA analysis to non-humans as ambitiously as it has been applied to our own species, making sure to treat ancient specimens in an ethical way that preserves them for future generations.

<sup>1</sup>Joukowsky Institute for Archaeology and the Ancient World, Brown University, Providence, RI 02912, USA

<sup>2</sup>Center for Computational Molecular Biology, Brown University,

**Box 1. Sources of aDNA, Temporal Constraints, and Ecological Zones Available for Study**

Most archaeogenomic research relies on aDNA extracted from bones excavated from archaeological sites. For non-human organisms, there are many substrates in addition to bones that can be targeted for aDNA extraction [89]. Potential sources of aDNA include archaeological artifacts; paleontological specimens and archaeological ecofacts (remains of biological materials found at archaeological sites); natural history, ethnographic, and archival collections; and soils (Table 1). Many of these materials also provide rich contextual information that can help with the interpretation of archaeogenomic data, making them important resources for future aDNA studies.

Despite a wide range of potential sources of aDNA data, there are spatial and temporal limits to aDNA research. To date, the oldest ancient genome comes from an approximately 700 000-year-old horse bone preserved in permafrost [90]. It is unlikely that DNA will preserve in samples that are much older than this, which means aDNA can only be used to study animals and plants that lived during the Middle Pleistocene and especially during the Late Pleistocene and the Holocene. The time periods available for study are even more restricted when working with natural history and museum specimens that represent flora and fauna collected during the past few hundred years. We still do not fully understand the processes that lead to DNA degradation in various types of materials, but preservation conditions in bone seem anecdotally to be better than in other soft tissues from plants and animals, even when they are somewhat intact such as in natural or anthropogenic mummies. As a result, it may be more difficult to obtain very old DNA from plants than animals. While age, temperature, and microbial attack are major factors that destroy DNA [1,91], it is unclear how other factors, especially storage conditions for materials after excavation and when they enter museum collections, contribute to DNA destruction. Therefore, it may not always be possible to extract aDNA from otherwise well-preserved archaeological, ethnographic, and natural history collections.

The types of ancient habitats that can be studied using aDNA are also limited because of unequal DNA preservation under different environmental conditions. DNA survival depends on thermal age, with higher levels of DNA preservation in temperate and cold environments than in hot equatorial climates [1]. We may be able to learn a great deal about plants and animals that lived in arctic environments, but relatively less about ecological diversity in ancient desert or tropical ecosystems at lower latitudes. For animals with large ranges that extend across multiple environmental zones, high resolution analysis may therefore be much easier for subpopulations that lived in the cooler parts of their original range. Encouragingly, recent technical improvements in aDNA analysis (including more efficient methods of extraction of DNA [92], conversion of the DNA into a form that can be sequenced [93], and the realization that the inner ear region of the petrous bone can preserve up to 100 times more DNA than other skeletal elements [94,95]) have made aDNA analysis in humans sufficiently efficient that high-quality data are now regularly being obtained from hot or tropical regions [96–99], and from Middle Pleistocene [100] and Late Pleistocene material [101] in temperate zones. It is reasonable to think that application of similar approaches will enable paleogenomic research in warm regions for non-human species as well.

Many of the technological breakthroughs and contributions of paleogenomics to evolutionary genomics have been described in recent reviews [1–6]. These reviews describe how genome-wide aDNA from well-dated samples (going beyond the studies of one or a few genetic locations such as mitochondrial DNA or chloroplast DNA that characterized the field of aDNA for its first few decades) makes it possible to track genetic continuity or change through time. Paleogenomics lets us document evolution as it happened and collect time-series data on evolution and selection. It is now possible to examine how selection shapes genomes over extended spans of time, how migration and admixture events produced current patterns of genetic variation, how species acquired genetic variants that provided fitness advantages under new selection constraints, and how plant and animal populations respond to environmental change, disease, or human activities.

To highlight the untapped potential of paleogenomic research in non-human organisms, we discuss three recent case studies on the evolutionary history and extinction of cave bears [7], the domestication of horses [8], and the domestication of maize [9–13]. These examples show the power of paleogenomic methods for informing paleoecological, paleoenvironmental, and archaeological questions about past species. We also discuss challenges and opportunities for the future of paleogenomic research on non-human organisms.

Two out of the three examples we focus on here are specifically archaeogenomic: genome-wide studies of aDNA found in association with ancient humans and with ample archaeological

Providence, RI, 02912, USA

<sup>3</sup>Department of Genetics, Harvard Medical School, Boston, MA 02115, USA

<sup>4</sup>Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

<sup>5</sup>Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA

\*Correspondence:  
[katherine\\_brunson@brown.edu](mailto:katherine_brunson@brown.edu)  
(K. Brunson).

context. There is of course also much to be learned from paleogenomic studies of paleontological, herbarium, and other ancient specimens collected outside of archaeological contexts, but we have chosen to focus on archaeogenomic case studies for the most part because we wish to highlight how ancient genomic data from non-humans helps to reveal the ways that humans have interacted with other species, modified past ecosystems and environments, and selected for traits in domesticated taxa. We also limit our discussion to plants and animals. aDNA from microbes and pathogens found in human bones and dental calculus is profoundly expanding our capacity to study human health and disease [14–17]. Pathogens also have been characterized from non-human samples such as plant materials [18–20], and many of the issues raised here about the future of archaeogenomic research also apply to these organisms.

### Case Study #1: Cave Bear Genomes Shed Light on Their History and Extinction

Cave bears (*Ursus spelaeus* complex) are one of the most extensively studied non-human species in paleogenomic research, and as such provide a case example of the promise of these approaches when applied more broadly (see Knapp [21] for a recent review). In 2018, Barlow *et al.* [7] published a study of complete cave bear genomes that provides substantial new insight into the evolution and history of this extinct species. The authors sequenced four European cave bear genomes, dated to about 72 000–35 000 years ago, and one brown bear (*Ursus arctos*) genome, dated to about 41 000 years ago. Comparisons with modern bear genomes confirmed the hypothesis based on mtDNA that cave bears are a sister clade of modern brown bears and polar bears [22,23]. Leveraging the power of their genome-wide data, the authors also documented that there was substantial ancient admixture between cave bears and brown bears after the initial population separation, a pattern that cannot be appreciated based on the single assessment of genealogical history provided by mtDNA. Although cave bears went extinct by about 25 000 years ago [24], they are estimated to have contributed 0.9–1.8% of the genomes of brown bear populations living today. The proportion of cave bear ancestry in the genome of the ancient brown bear was even higher, reaching 2.4%. These results support growing evidence that ancient admixture is common in evolution [25]. Mixture of this type between closely related taxa seems to be a common phenomenon in biology [26,27], including among bears [28–30].

aDNA research, primarily drawn from mtDNA, has also been used to shed light on the potential causes of cave bear extinction. Numerous species of megafauna went extinct at the end of the Pleistocene [31–35]. The degree to which humans contributed to these extinctions is debated [36], but it is broadly accepted that many species, including cave bears, were impacted by a combination of environmental and anthropogenic factors [33,35,37,38]. Studies of the biogeographic distributions of cave bear mtDNA lineages show that they were impacted by climate change during the Late Pleistocene. For example, cave bear ranges contracted toward the end of the Last Glacial Maximum [35], with animals surviving in more isolated regions for the longest time [24].

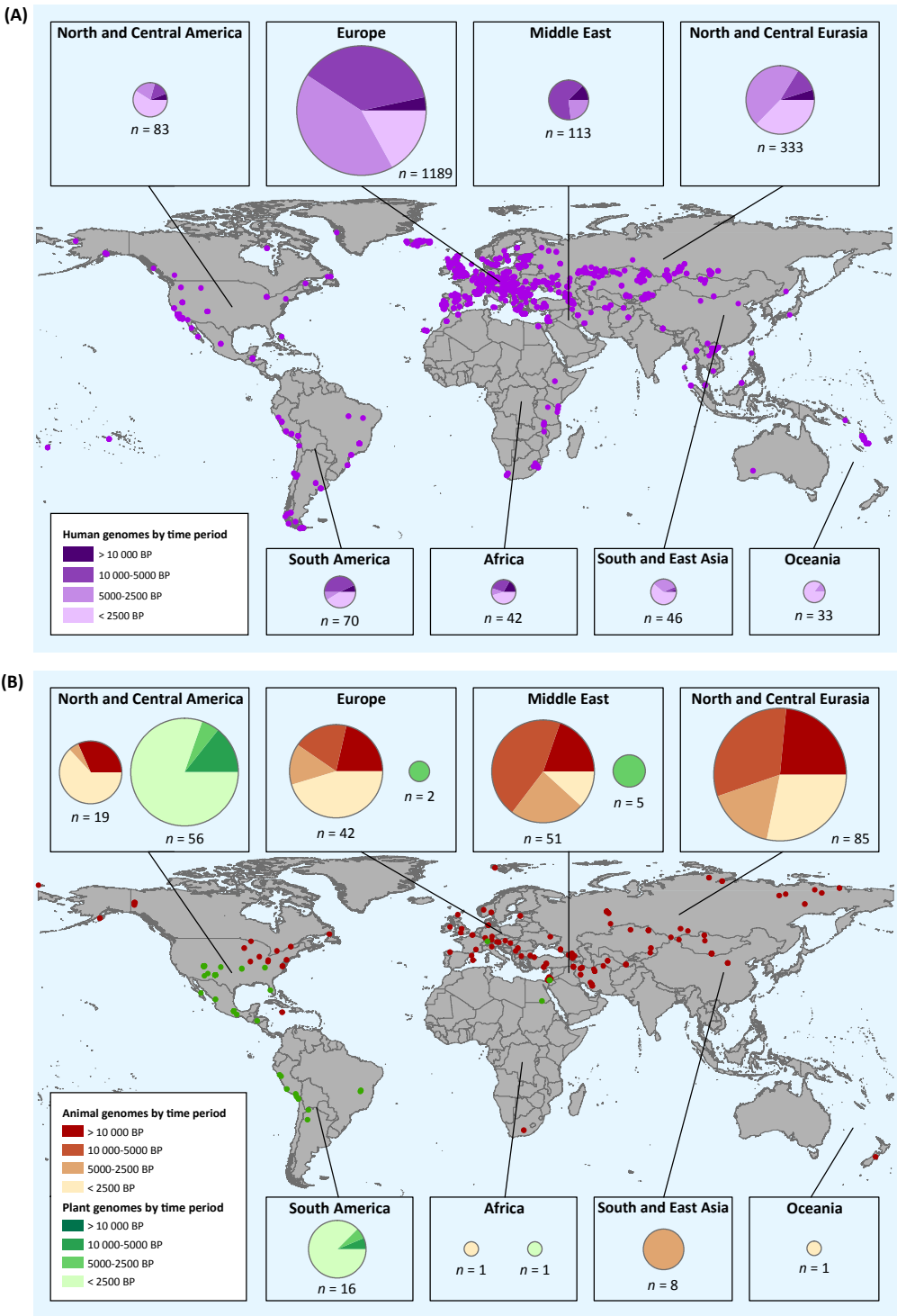
Cave bears may have also been more vulnerable to human predation and competition for habitats than other bear species, including brown bears that did not go extinct. Stiller *et al.* [38] found that European cave bear population sizes decreased significantly over the 25 000 years prior to their extinction, while brown bear populations did not experience the same dramatic collapse. They proposed that the different hibernation strategies of cave bears and brown bears (cave bears hibernate in caves whereas brown bears hibernate in a variety of locations) may have put cave bears in direct competition with humans for access to cave sites. Fortes *et al.* [37] came to a similar conclusion after studying cave bear and brown bear mitochondrial genomes

### Glossary

**Ancient DNA (aDNA):** DNA extracted and sequenced from the remains of an organism long after it has died; usually ‘ancient’ refers to samples over 100 years old.

**Archaeogenomics:** paleogenomic research on samples excavated from archaeological sites and analyzed in relation to their archaeological (human) context.

**Paleogenomics:** the analysis of aDNA drawn from a sufficient number of locations in ancient genomes to obtain a statistically meaningful assessment of patterns (this can be as few as hundreds of locations, but can often mean millions or even billions of sequences).



Trends in Genetics

Figure 1. Geographic and Temporal Distribution of Ancient Genomes Published through December 15, 2018. Marciniak and Perry [3] previously reported a map of published human sequences. Here we generate an enhanced version of this figure, that reports both published genomic data from (A) human individuals (points in purple, updated from Marciniak and Perry,  $n = 1909$ ), and (B) non-human animal (red,  $n = 207$ ) and plant (green,  $n = 80$ ) species. For the human samples, we restrict

(Figure legend continued on the bottom of the next page.)

Table 1. Sources of aDNA from Non-Human Organisms

Material	Source of aDNA	Temporal range	Examples
Archaeological artifacts	DNA from residues on artifact surfaces or from resins, glues, and other materials used in artifact construction.	<700 kya	Milk residues on a ceramic pot used to heat goat milk. Animal blood residues on the edge of a stone tool used for butchering meat.
Paleontological specimens and archaeological ecofacts	Plant and animal DNA preserved in the physical remains of the organism themselves.	<700 kya	Fur from a mammoth preserved in permafrost. Animal bones and remains of plants consumed by people who lived at an archaeological site. Coprolites from animals that lived in a cave.
Natural history, herbaria, ethnographic, and archival collections	Plant and animal DNA preserved in the remains of the organism themselves.	Generally restricted to materials collected during biological and anthropological fieldwork within the past 200 years.	Herbaria collections. Taxidermy animals. Book parchment in library collections. Ethnographic objects made from feathers, skin, fur, shell, and plant materials.
Soils	DNA preserved in soils when no other physical remains of the organism survive.	<700 kya	Environmental DNA from lake sediment cores.

from northern Spain. Cave bear bones belonged to distinct mitochondrial lineages of closely related haplotypes that were cave-specific, suggesting that cave bears hibernated communally and frequented the same cave sites where they were born. Homing behavior may have made this species less flexible when they crossed paths with humans living in caves, putting them at risk.

A limitation of Stiller *et al.* and Fortes *et al.*'s reconstructions of cave bear demographic history, like most aDNA studies of non-humans to date, was that they exclusively analyzed mtDNA variation. While this is a starting point, it only provides information about the maternal lineage, and genome-wide data which involves assessment of enough independent locations in the genome to make statistically meaningful statements are needed to make precise reconstructions of demographic events. As paleogenomic research in humans, which first began to be published in 2010, has shown, conclusions based on mtDNA analyses are often challenged by genomic data. For example, Neanderthal mtDNA is distinct from that of modern humans, but autosomal DNA reveals that Neanderthals and humans interbred such that the genomes of non-Africans today still show about 2% Neanderthal ancestry [39,40]. We need genome-wide analysis to test hypotheses about cave bears and other extinct species originally inferred based on one or a few locations, and future genome-wide research will undoubtedly provide even greater power to clarify the causes of extinction. What we learn about ancient genomes may also help to identify interventions that can save threatened species today (see Outstanding Questions).

## Case Study #2: Ancient Horse Genomes Reveal Multiple Episodes of Domestication

Domestication involves mutualistic relationships between humans and domesticated plant and animal species; it occurs on a continuum ranging from minimally managed species requiring

---

to individuals with >0.025X coverage on a genome-wide set of informative single nucleotide polymorphism positions. For animals and plants, we restrict to samples in which multiple autosomal loci have been characterized. Samples with only mitochondrial or chloroplast genomes are not included. Data can be found in the online supplementary data. Maps drawn by Miriam Rothenberg in ArcGIS.

only limited human investment, to fully domesticated species that rely on humans for all aspects of their survival and reproduction [41]. Paleogenomic technology is changing our understanding of domestication as it gives us the opportunity to witness the process as it unfolds instead of being limited to studying the end product. Arguably the most impressive research in this area has been on horses, the species in which the quality of paleogenomic data and population genomic analysis has been most similar to, and in some cases has exceeded, that in humans. The startling findings of these studies highlight the potential of aDNA studies of non-humans.

The earliest archaeological evidence for horse domestication comes from sites in the Pontic-Caspian steppe and Kazakhstan, with the Eneolithic Botai culture dated to around 5500 years ago [42–45]. In the most ambitious aDNA study of non-humans to date, Gaunitz *et al.* [8] sequenced over 40 ancient horse genomes and found evidence for multiple horse domestication processes and an unanticipated feralization of the Przewalski's horse. The authors compared genomes from 20 horse samples associated with the Botai culture sites with genomes from 20 horse samples excavated from other locations in Europe and Central Asia, covering the past 5000 years. They also compared these ancient genomes with previously published ancient and modern domestic horse genomes, and a 19th century Przewalski's horse genome [43,46–48].

Przewalski's horses have long been considered to be the only living wild horse [47]. However, Gaunitz *et al.* found that the Przewalski's horses clustered together with the ancient domestic Botai horses, which is a pattern consistent with descending directly from them, while all other domestic horses clustered into a separate monophyletic group. The simplest explanation for this is that Przewalski's horses are a feral population descended from the horses that were first domesticated at sites like Botai. Modern domestic horses likely resulted from a separate later domestication episode that occurred sometime before 4000 years ago, from a different lineage of wild horses before the time that all wild horses went extinct.

The analysis of Gaunitz *et al.* also showed that there was admixture between various horse populations. This is another important piece of paleogenomic evidence that interbreeding between closely related populations is common. In some cases where ancient admixture has been identified, interbreeding appears to have introduced adaptive alleles into ancient populations [49–52]. For domesticated species, introgressive capture of wild animals and admixture between domestic and wild stocks was an important feature of animal exploitation [53–56]. Admixture with wild populations plausibly increased herd health by re-introducing genetic diversity to domestic populations and may have given domesticated species advantages when living in new environments [54,56–59].

The surprising findings of Gaunitz *et al.* about horses reveal that animal domestication can be a complex process, characterized by feralization, population turnover events, and interbreeding between wild and domestic stocks [53–56,60]. These findings underscore the point that past population dynamics cannot always be inferred from present-day DNA variation. This is especially the case for horses. All living Przewalski's horses descend from just over a dozen founder individuals captured during the turn of the 20th century and saved from extinction through captive-breeding programs [61]. Przewalski's horses and modern domestic horses may appear to be from unrelated lineages when modern DNA is analyzed, but paleogenomics reveals that they did not evolve in complete isolation, and that the history of horses may have involved multiple experiments in human management of wild populations that are difficult to detect without the insight provided by ancient genomes. Paleogenomics has also revealed the existence of archaic lineages of now-extinct horse populations that would have remained



unknown if we only conducted genomic studies of living horses [46]. Future aDNA studies will further clarify the evolutionary history of horses, including the social dimensions of domestication and selection (see Outstanding Questions).

### Case Study #3: Ancient Maize Genomes Reveal a Long-Drawn-Out Domestication Process

In general, plant remains do not preserve well in ancient contexts unless they are burned and carbonized, a process that destroys DNA which may explain why so few ancient plant genomes have been sequenced. However, some dry environments, especially dry caves in highland Mexico, have excellent preservation of uncarbonized plant materials, and corn cobs discarded in such environments are a particularly good preservation context for DNA. This has allowed for new insights on the domestication of maize.

Archaeological evidence indicates that maize was domesticated from the wild teosinte plant in southern Mexico by about 8700 years ago, with some of the earliest evidence in the form of starch grains and phytoliths found on the surfaces of stone tools [62]. Macrobotanical remains dated to over 5000 years ago show phenotypic changes associated with domestication. For example, ancient maize cobs contain larger kernels, more rows of kernels, and do not have hard coatings over the kernels [63]. Initial aDNA studies of maize were also able to target a limited number of loci known to be important in modern domesticated maize, revealing that alleles associated with kernel quality and cob size were already present 4400 years ago in maize cobs from Mexico [64]. Genome-wide aDNA studies have allowed researchers to go one step further to identify additional candidate loci under selection during domestication.

There have been several recent paleogenomic studies of maize [9–13]. Together, these studies reveal that phenotypic traits associated with modern domesticated maize emerged slowly and at different times during maize domestication. In separate studies, Ramos-Madriral *et al.* [11] and Vallebuena-Estrada *et al.* [13] each sequenced ~5000 year old maize cobs from the Tehuacán Valley of Mexico. Although these early cobs already had several traits of modern domestic maize, such as a brittle endosperm and naked kernels, the ancestral versions of many other alleles associated with domestic phenotypes were still present. For example, mutations associated with ear shattering and increased starch production were not present 5000 years ago. In their study of 32 ancient maize partial exomes (comprising 348 genes of interest) spanning the past 6000 years, da Fonseca *et al.* [9] examined later selection events associated with the emergence of some of these derived traits. Many of the alleles found in modern maize landraces, such as those that contribute to increased starch production, emerged only in the past 2000 years as maize was introduced to new geographic regions. In particular, as maize was introduced to dry regions of the United States Southwest ~2000–750 years ago, humans selected for alleles associated with drought tolerance and starch production, which may have helped people grow and store maize in these environments. More recent research by Swarts *et al.* [12] has further confirmed that temperate-adapted maize was subject to gradual selection *in situ* by ancestral Puebloan peoples in the United States. Kistler *et al.* [10] studied ancient maize genomes from Peru and Brazil and revealed that wild type variants persisted in South American maize lineages, suggesting that when maize was introduced into South America ~6500 years ago it did not yet contain all of the alleles associated with modern maize varieties. Further domestication of maize occurred in parallel in North and South America.

These studies highlight how selection for phenotypic traits associated with domestication can occur slowly. Geneticists working with modern datasets often identify selective sweeps to determine candidate ‘domestication genes’ that were subject to selection during plant and

animal domestication [65,66]. However, these sweeps may be the product of the past few hundred years of directed breeding and recent admixture, and do not always identify earlier selection events associated with initial domestication [67]. The most powerful way to determine when selection events took place and when phenotypes associated with domestic taxa became common is by using time-series genomic data from before and after these important events [6].

### Concluding Remarks and Future Perspectives

The case studies above reveal that the unique behaviors and ecologies of different species may make them more or less vulnerable to extinction, and that domestication is a complicated process characterized by feralization, admixture, and gradual selection on phenotypic traits. These insights from paleogenomics provide new insights into evolutionary processes and the ways that human activities have impacted other species. They also demonstrate the value of genome-wide data over studies of single genetic loci. Cave bears, horses, and maize are some of the most heavily researched species other than humans, and if we wish to realize the full potential of paleogenomics to examine evolutionary and genetic processes for earth's organisms, it will be important to expand such studies to many more species. There are a number of issues that require attention:

- 1) It is important to continue to adapt aDNA technology to extend the reach of this research to more taxa (see [4] for a summary of key technological developments in paleogenomics). Compiling and selecting appropriate modern reference genomes to use while reconstructing ancient genomes is a major challenge, especially when working on species without long histories of research. One recent trend has been to shift from brute-force 'shotgun' sequencing of entire ancient genomes to using targeted capture approaches that only amplify SNPs of interest. This approach makes aDNA analysis more cost-effective and has transformed human aDNA research; as a concrete example of this, targeted capture data is now responsible for 62% of published human samples with genome-wide data (see online supplementary data). However, to make this technology available beyond humans, there will have to be up-front investment in laboratory reagents for targeting variation in each species of interest. In addition, there may be strong ascertainment biases inherent in targeted capture approaches, because for many species, targeting SNPs based on modern DNA variation will not provide an accurate picture of past genetic diversity. This is especially likely when studying domesticated plants and animals because modern breeds and landraces have been modified significantly during the past few hundred years of directed breeding and selection for use in modern industrialized agricultural practices.
- 2) We must be careful and ethical stewards of ancient biological resources. Museums and archaeological repositories provide important archives of past biological diversity. The destruction of the National Museum of Brazil in September 2018 is a reminder of the irreplaceable value of many natural history and anthropological collections. It is important to remember that these collections, as well as remains that come from active archaeological excavations, are finite resources. There have been recent calls to implement best practices for ethical sampling of human remains for aDNA research [68,69], and attention to these issues is needed for non-human samples as well [70]. Guidelines for ethical access and sharing of modern genetic resources [71] should be extended in an appropriate way to ancient biological samples. Productive and continuous dialogue is necessary between paleogeneticists, museum staff, archaeologists, local communities, and other stakeholders. When destructive sampling is used on material that is morphologically unique (that is, there are not morphologically similar specimens remaining in collections), it should be done in combination with careful documentation of the original specimen's morphology prior to sampling, preferably using 3D scanning or photogrammetry so that replicas can still be

### Outstanding Questions

How can genome-wide data from a greater variety of species help us to better understand biology? For example, how can we refine species-specific molecular clock estimates, or document how gene expression pathways changed during domestication (using the epigenetic information preserved in aDNA)?

In addition to clarifying causes of Late-Pleistocene extinctions for animals such as cave bears (case study #1) and other species such as woolly mammoths [80,81], how can aDNA help document extinctions associated with the arrival of humans into new regions such as islands [82,83], or recent extirpations and extinctions associated with habitat destruction during the expansion of agriculture [84]? What roles should aDNA have in modern efforts to fight extinction [85], and how can we use paleogenomic data to inform modern conservation biology or predict species-specific responses to climate change [86,87]?

What was the role of feralization, introgressive capture of phenotypically important genetic variants, and admixture in the histories of domesticated species? Which gene variants were subject to selection during initial domestication events and which were selected later as domestic species were introduced into new environments or used in new cultural settings?

How can aDNA contribute to our understanding of the social dimensions of domestication? For example, how might the identification of phenotypic traits such as coat color reveal ancient cultural preferences [43]?

How can we use paleogenomics and specifically archaeogenomics to improve agricultural crops by re-introducing ancient traits [88]? How can archaeogenomics inform modern agricultural practices to make them more sustainable?

How can we continue to build interdisciplinary collaborations that will improve paleogenomic research design, ensure that aDNA samples are collected ethically, and lead to more sophisticated interpretations of aDNA data?



studied. aDNA analyses typically require sample sizes of 10–100 mg, which is substantially less than the 100–1000 mg required for radiocarbon dating, although balanced against this is the fact that aDNA studies sometimes necessitate destruction of morphologically more informative specimens (such as petrous bones in the case of mammals). Nevertheless, aDNA research should continue to develop minimally destructive methods [72]. One potentially important untapped resource is soils and sediment cores with stratigraphically distinct layers. Metagenomic environmental DNA from sediment cores can show temporal changes in ecological diversity even when physical remains of those species do not preserve [73,74]. However, the amounts of DNA from vertebrate species of interest that emerge from metagenomic analysis are typically much less than that obtained from direct sampling of remains from the organisms, so we expect that destructive analysis will continue to play an important role in paleogenomic research.

- 3) Paleogenomics must be fully incorporated into evolutionary theory. aDNA from humans has already caused paradigm shifts in genomics research by revealing the significant role of admixture in past population dynamics [75]. The work that has been published so far on non-human organisms is only the beginning, and future research will undoubtedly force us to revise our understanding of evolutionary processes. Epigenetics is one area that is promising for new discovery [2,4,76–78], as is using microbial DNA from bones, teeth, and dental calculus, which can be used to examine ancient microbiomes, zoonotic diseases, and more [16,17,79].
- 4) Finally, it is important to continue to build collaborations between geneticists and researchers working in other disciplines, such as archaeology and environmental studies, in order to ensure that paleogenomic results are interpreted within their appropriate cultural, historical, and environmental contexts.

### Acknowledgements

Three anonymous reviewers provided suggestions that greatly improved the manuscript. The paper also benefited from discussions with members of the Ramachandran Laboratory at Brown University. K.B. is supported in part by NSF CAREER DBI-1452622. K.B. would like to acknowledge the Joukowsky Institute for Archaeology and the Ancient World and the Center for Computational Molecular Biology at Brown University. D.R. is an Investigator of the Howard Hughes Medical Institute.

### Supplemental Information

Supplemental information associated with this article can be found online at <https://doi.org/10.1016/j.tig.2019.02.006>.

### References

1. Hofreiter, M. *et al.* (2014) The future of ancient DNA: technical advances and conceptual shifts. *BioEssays*, 37, 284–293
2. Leonardi, M. *et al.* (2017) Evolutionary patterns and processes: lessons from ancient DNA. *Syst. Biol.* 66, e1–e29
3. Marciniak, S. and Perry, G.H. (2017) Harnessing ancient genomes to study the history of human adaptation. *Nat. Rev. Genet.* 18, 659
4. Orlando, L. *et al.* (2015) Reconstructing ancient genomes and epigenomes. *Nat. Rev. Genet.* 16, 395
5. Shapiro, B. and Hofreiter, M. (2014) A paleogenomic perspective on evolution and gene function: new insights from ancient DNA. *Science*, 343, 1236573
6. Malaspinas, A.-S. (2016) Methods to characterize selective sweeps using time serial samples: an ancient DNA perspective. *Mol. Ecol.* 25, 24–41
7. Barlow, A. *et al.* (2018) Partial genomic survival of cave bears in living brown bears. *Nat. Ecol. Evol.* 2, 1563–1570
8. Gauntitz, C. *et al.* (2018) Ancient genomes revisit the ancestry of domestic and Przewalski's horses. *Science*, 360, 111–114
9. da Fonseca, R.R. *et al.* (2015) The origin and evolution of maize in the Southwestern United States. *Nat. Plants*, 1, 14003
10. Kistler, L. *et al.* (2018) Multiproxy evidence highlights a complex evolutionary legacy of maize in South America. *Science*, 362, 1309–1313
11. Ramos-Madrigal, J. *et al.* (2016) Genome sequence of a 5,310-year-old maize cob provides insights into the early stages of maize domestication. *Curr. Biol.* 26, 3195–3201
12. Swarts, K. *et al.* (2017) Genomic estimation of complex traits reveals ancient maize adaptation to temperate North America. *Science*, 357, 512–515
13. Vallebuena-Estrada, M. *et al.* (2016) The earliest maize from San Marcos Tehuacán is a partial domesticate with genomic evidence of inbreeding. *Proc. Natl. Acad. Sci. U. S. A.* 113, 14151–14156
14. Warinner, C. *et al.* (2017) A robust framework for microbial archaeology. *Annu. Rev. Genomics Hum. Genet.* 18, 321–356
15. Warinner, C. *et al.* (2015) Ancient human microbiomes. *J. Hum. Evol.* 79, 125–136

16. Vågåne, Å.J. *et al.* (2018) *Salmonella enterica* genomes from victims of a major sixteenth-century epidemic in Mexico. *Nat. Ecol. Evol.* 2, 520–528
17. Rasmussen, S. *et al.* (2015) Early divergent strains of *Yersinia pestis* in Eurasia 5,000 years ago. *Cell*, 163, 571–582
18. Martin, M.D. *et al.* (2013) Reconstructing genome evolution in historic samples of the Irish potato famine pathogen. *Nat. Commun.* 4, 2172
19. Yoshida, K. *et al.* (2013) The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. *eLife*, 2, e00731
20. Smith, O. *et al.* (2014) A complete ancient RNA genome: identification, reconstruction and evolutionary history of archaeological barley stripe mosaic virus. *Sci. Rep.* 4, 4003
21. Knapp, M. (2018) From a molecules' perspective – contributions of ancient DNA research to understanding cave bear biology. *Hist. Biol.* 31, 1–6
22. Noonan, J.P. *et al.* (2005) Genomic sequencing of Pleistocene cave bears. *Science*, 309, 597–599
23. Krause, J. *et al.* (2008) Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene–Pliocene boundary. *Evol. Biol.* 8, 220
24. Baca, M. *et al.* (2016) Retreat and extinction of the Late Pleistocene cave bear (*Ursus spelaeus sensu lato*). *Sci. Nat.* 103, 11–12
25. Prüfer, K. *et al.* (2013) The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature*, 505, 43
26. Harrison, R.G. and Larson, E.L. (2014) Hybridization, introgression, and the nature of species boundaries. *J. Hered.* 105, 795–809
27. Shurtliff, Q.R. (2013) Mammalian hybrid zones: a review. *Mamm. Rev.* 43, 1–21
28. Cahill, J.A. *et al.* (2015) Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Mol. Ecol.* 24, 1205–1217
29. Kumar, V. *et al.* (2017) The evolutionary history of bears is characterized by gene flow across species. *Sci. Rep.* 7, 46487
30. Miller, W. *et al.* (2012) Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proc. Natl. Acad. Sci. U. S. A.* 109, E2382–E2390
31. Barnosky, A.D. *et al.* (2004) Assessing the causes of Late Pleistocene extinctions on the continents. *Science*, 306, 70–75
32. Chang, D. *et al.* (2017) The evolutionary and phylogeographic history of woolly mammoths: a comprehensive mitogenomic analysis. *Sci. Rep.* 7, 44585
33. Lorenzen, E.D. *et al.* (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature*, 479, 359
34. Palkopoulou, E. *et al.* (2013) Holarctic genetic structure and range dynamics in the woolly mammoth. *Proc. R. Soc. B Biol. Sci.* 280, 20131910
35. Stiller, M. *et al.* (2014) Mitochondrial DNA diversity and evolution of the Pleistocene cave bear complex. *Quat. Int.* 339–340, 224–231
36. Meltzer, D.J. (2015) Pleistocene overkill and North American mammalian extinctions. *Annu. Rev. Anthropol.* 44, 33–53
37. Fortes, G.G. *et al.* (2016) Ancient DNA reveals differences in behaviour and sociality between brown bears and extinct cave bears. *Mol. Ecol.* 25, 4907–4918
38. Stiller, M. *et al.* (2010) Withering away—25,000 years of genetic decline preceded cave bear extinction. *Mol. Biol. Evol.* 27, 975–978
39. Green, R.E. *et al.* (2010) A draft sequence of the Neandertal genome. *Science*, 328, 710–722
40. Sankararaman, S. *et al.* (2012) The date of interbreeding between Neandertals and modern humans. *PLoS Genet.* 8, e1002947
41. Zeder, M.A. *et al.* (2012) Pathways to animal domestication. In *Biodiversity in Agriculture: Domestication, Evolution, and Sustainability* (Gepts, P., ed.), pp. 227–259, Cambridge University Press
42. Anthony, D.W. (2007) *The Horse, the Wheel, and Language: How Bronze-Age Riders from the Eurasian Steppes Shaped the Modern World*, Princeton University Press
43. Librado, P. *et al.* (2017) Ancient genomic changes associated with domestication of the horse. *Science*, 356, 442–445
44. Olsen, S.L. *et al.* (2006) Early horse domestication on the Eurasian Steppe. In *Documenting Domestication: New Genetic and Archaeological Paradigms* (Zeder, M.A., ed.), pp. 245–269, University of California Press
45. Outram, A.K. *et al.* (2009) The earliest horse harnessing and milking. *Science*, 323, 1332–1335
46. Schubert, M. *et al.* (2014) Prehistoric genomes reveal the genetic foundation and cost of horse domestication. *Proc. Natl. Acad. Sci. U. S. A.* 111, E5661–E5669
47. Der Sarkissian, C. *et al.* (2015) Evolutionary genomics and conservation of the endangered Przewalski's horse. *Curr. Biol.* 25, 2577–2583
48. Librado, P. *et al.* (2015) Tracking the origins of Yakutian horses and the genetic basis for their fast adaptation to subarctic environments. *Proc. Natl. Acad. Sci. U. S. A.* 112, E6889–E6897
49. Enard, D. and Petrov, D.A. (2018) Evidence that RNA viruses drove adaptive introgression between Neanderthals and modern humans. *Cell*, 175, 360–371
50. Dannemann, M. *et al.* (2016) Introgression of Neandertal- and Denisovan-like haplotypes contributes to adaptive variation in human toll-like receptors. *Am. J. Hum. Genet.* 98, 22–33
51. Huerta-Sánchez, E. *et al.* (2014) Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature*, 512, 194
52. Racimo, F. *et al.* (2015) Evidence for archaic adaptive introgression in humans. *Nat. Rev. Genet.* 16, 359
53. Larson, G. and Burger, J. (2013) A population genetics view of animal domestication. *Trends Genet.* 29, 197–205
54. Larson, G. and Fuller, D.Q. (2014) The evolution of animal domestication. *Annu. Rev. Ecol. Evol. Syst.* 45, 115–136
55. MacHugh, D.E. *et al.* (2017) Taming the past: ancient DNA and the study of animal domestication. *Annu. Rev. Anim. Biosci.* 5, 329–351
56. Marshall, F.B. *et al.* (2014) Evaluating the roles of directed breeding and gene flow in animal domestication. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6153–6158
57. Hedrick, P.W. (2013) Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol. Ecol.* 22, 4606–4618
58. Miao, B. *et al.* (2017) Genomic analysis reveals hypoxia adaptation in the Tibetan Mastiff by introgression of the gray wolf from the Tibetan Plateau. *Mol. Biol. Evol.* 34, 734–743
59. Skoglund, P. *et al.* (2015) Ancient wolf genome reveals an early divergence of domestic dog ancestors and admixture into high-latitude breeds. *Curr. Biol.* 25, 1515–1519
60. Ottoni, C. *et al.* (2012) Pig domestication and human-mediated dispersal in western Eurasia revealed through ancient DNA and geometric morphometrics. *Mol. Biol. Evol.* 30, 824–832
61. Goto, H. *et al.* (2011) A massively parallel sequencing approach uncovers ancient origins and high genetic variability of endangered Przewalski's horses. *Genome Biol. Evol.* 3, 1096–1106
62. Piperno, D.R. *et al.* (2009) Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico. *Proc. Natl. Acad. Sci. U. S. A.* 106, 5019–5024
63. Staller, J. *et al.* (2006) In *Histories of Maize: Multidisciplinary Approaches to the Prehistory, Linguistics, Biogeography, Domestication, and Evolution of Maize*, Academic Press

64. Jaenicke-Després, V. *et al.* (2003) Early allelic selection in maize as revealed by ancient DNA. *Science*, 302, 1206–1208
65. Axelsson, E. *et al.* (2013) The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature*, 495, 360–364
66. Rubin, C.-J. *et al.* (2010) Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*, 464, 587
67. Flink, L.G. *et al.* (2014) Establishing the validity of domestication genes using DNA from ancient chickens. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6184–6189
68. Makarewicz, C. *et al.* (2017) Ensure equal access to ancient DNA. *Nature*, 548, 158
69. Prendergast, M.E. and Sawchuk, E. (2018) Boots on the ground in Africa's ancient DNA 'revolution': archaeological perspectives on ethics and best practices. *Antiquity*, 92, 803–815
70. Austin, R.M. *et al.* (2019) Opinion: to curate the molecular past, museums need a carefully considered set of best practices. *Proc. Natl. Acad. Sci. U. S. A.* 116, 1471–1474
71. Convention on Biological Diversity (2011) Nagoya protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization to the Convention on Biological Diversity, United Nations
72. Sirak, K.A. *et al.* (2017) A minimally-invasive method for sampling human petrous bones from the cranial base for ancient DNA analysis. *BioTechniques*, 62, 283–289
73. Thomsen, P.F. and Willerslev, E. (2015) Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* 183, 4–18
74. Slon, V. *et al.* (2017) Neandertal and Denisovan DNA from Pleistocene sediments. *Science*, 356, 605–608
75. Pickrell, J.K. and Reich, D. (2014) Toward a new history and geography of human genes informed by ancient DNA. *Trends Genet.* 30, 377–389
76. Gokhman, D. *et al.* (2016) Epigenetics: it's getting old. Past meets future in paleoepigenetics. *Trends Ecol. Evol.* 31, 290–300
77. Pedersen, J.S. *et al.* (2014) Genome-wide nucleosome map and cytosine methylation levels of an ancient human genome. *Genome Res.* 24, 454–466
78. Hanghoj, K. *et al.* (2016) Fast, accurate and automatic ancient nucleosome and methylation maps with epiPALEOMIX. *Mol. Biol. Evol.* 33, 3284–3298
79. Warinner, C. *et al.* (2015) A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Philos. Trans. R. Soc. B Biol. Sci.* 370, 20130376
80. Palkopoulou, E. *et al.* (2015) Complete genomes reveal signatures of demographic and genetic declines in the woolly mammoth. *Curr. Biol.* 25, 1395–1400
81. Rogers, R.L. and Slatkin, M. (2017) Excess of genomic defects in a woolly mammoth on Wrangel island. *PLoS Genet.* 13, e1006601
82. Grayson, D.K. (2001) The archaeological record of human impacts on animal populations. *J. World Prehist.* 15, 1–68
83. Seersholm, F.V. *et al.* (2018) Subsistence practices, past biodiversity, and anthropogenic impacts revealed by New Zealand-wide ancient DNA survey. *Proc. Natl. Acad. Sci. U. S. A.* 115, 7771–7776
84. Lander, B. and Brunson, K. (2018) Wild mammals of ancient North China. *J. Chin. Hist.* 2, 291–312
85. Shapiro, B. (2014) *How to Clone a Mammoth — the Science of De extinction*, Princeton University Press
86. Hofman, C.A. *et al.* (2015) Conservation archaeogenomics: ancient DNA and biodiversity in the Anthropocene. *Trends Ecol. Evol.* 30, 540–549
87. Leonard, J.A. (2008) Ancient DNA applications for wildlife conservation. *Mol. Ecol.* 17, 4186–4196
88. Di Donato, A. *et al.* (2018) Genome sequencing of ancient plant remains: findings, uses and potential applications for the study and improvement of modern crops. *Front. Plant Sci.* 9, 441
89. Green, E. and Speller, C. (2017) Novel substrates as sources of ancient DNA: prospects and hurdles. *Genes*, 8, 180
90. Orlando, L. *et al.* (2013) Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. *Nature*, 499, 74
91. Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature*, 362, 709
92. Dabney, J. *et al.* (2013) Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U. S. A.* 110, 15758–15763
93. Meyer, M. *et al.* (2012) A high-coverage genome sequence from an archaic Denisovan individual. *Science*, 338, 222–226
94. Pinhasi, R. *et al.* (2015) Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS One*, 10, e0129102
95. Gamba, C. *et al.* (2014) Genome flux and stasis in a five millennium transect of European prehistory. *Nat. Commun.* 5, 5257
96. Lazaridis, I. *et al.* (2016) Genomic insights into the origin of farming in the ancient Near East. *Nature*, 536, 419
97. Skoglund, P. *et al.* (2016) Genomic insights into the peopling of the Southwest Pacific. *Nature*, 538, 510
98. Lipson, M. *et al.* (2018) Ancient genomes document multiple waves of migration in Southeast Asian prehistory. *Science*, 361, 92–95
99. McColl, H. *et al.* (2018) The prehistoric peopling of Southeast Asia. *Science*, 361, 88–92
100. Meyer, M. *et al.* (2016) Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins. *Nature*, 531, 504
101. Hajdinjak, M. *et al.* (2018) Reconstructing the genetic history of late Neanderthals. *Nature*, 555, 652