# Bringing Back Extinct Species[a]

**George E. Seidel. Jr.**
**Colorado State University**

## Background

For a variety of reasons, people are fascinated by the possibility of recreating extinct species. Some feel guilty because our species has caused species to go extinct, such as the dodo, passenger pigeon, mammoths, and aurochs. There are science fiction and philosophical aspects exemplified in the book and film Jurassic Park, and there are ethical debates based on arguments like the absence of a suitable ecological niche for the re-created species, and the perceived waste of resources that otherwise could be applied to more pressing problems. Herein, I will limit myself primarily to issues of scientific feasibility, easily justified as intriguing teaching models illustrating scientific principles; also, such thinking might be applied to practical and theoretical biological situations. Any entertainment value is a bonus.

One problem is defining species, an issue that has been debated for decades. One reasonable definition is: a group of organisms that for practical purposes reproduces amongst themselves resulting in phenotypic differences from other species.

## Resources

In addition to imagination, we have an exceptional suite of tools such as assisted reproduction technologies that can be used to recreate extinct species. An enormous body of scientific literature is another major asset. There also are numerous remnants of extinct species, including frozen or mummified carcasses, snippets of DNA associated with or parts of the carcasses, and genetic information from closely related species that still survive (2). From the related species, we can infer much of the genetic information of the extinct species that is otherwise unavailable, and individuals of these species might, for example, serve as recipients for embryos and/or provide oocytes to function as factories for the DNA from extinct species. What we do not have is live cells from extinct species, except for several species very recently extinct (16), nor do we have reasonably intact cell nuclei or un-degraded components of DNA that would be suitable for cloning by somatic cell nuclear transfer.

There is, however, one cell type with a reasonable chance of surviving permafrost temperatures, with reasonably intact DNA: the sperm. Sperm DNA is highly condensed and relatively inert. Damage to DNA due to peroxidation and other chemical reactions would be much less than for other cell types, and the target volume for radiation damage is minimized. DNA damage still accumulates under these conditions from internal $^{40}K$ and cosmic rays. Damage might not be too severe over the span of 10,000 years, but likely would be problematic after 100,000 years. DNA repair enzymes in the oocyte (14) might be able to restore 10,000 years of radiation damage. Also promising are experiments simulating up to 2000 years of background radiation to mouse embryos at liquid nitrogen temperatures; such embryos resulted in normal offspring when thawed and transferred to reproductive tracts of recipients (6)

[a] First published as “recreating Extinct Species”, 2016, pp 81-85 in 26th Technical Conference on Artificial Insemination and Reproduction. National Association of Animal Breeders, Madison, WI.

If sperm were found in the epididymis of an extinct species frozen in permafrost, there would be many options available, with half of the sperm carrying an X chromosome and half a Y chromosome.

**Choice of species to recreate**

More probably has been written about recreating the wooly mammoth than all other mammalian species combined. This may be partly due to occasional finds of mammoth carcasses that are quite well preserved due to being frozen for centuries in permafrost in the arctic (12). Thus, tissue is available, composed of protein and likely containing considerable DNA. Elephants appear to be closely related to mammoths, and thus may have compatible ooplasm and mitochondria so that elephant oocytes may be of value for a project concerning mammoths. The elephant uterus may also be capable of gestating a mammoth under some circumstances, with likely similar gestation lengths for the two species. One trick to overcome an incompatible uterus gestating a conceptus of a closely related species (as occurs with sheep and goats), is to make chimeric embryos by mixing asynchronous blastomeres so that one effectively has the fetus of the incompatible species in the placenta of the recipient species, which then results in term pregnancies as has been done with sheep and goats (5).

The mammoth/elephant combination has obvious, serious limitations such as costs of maintaining a herd of elephant recipients, especially feed, late age at puberty (similar to humans), and 22-month gestation. The sabre-tooth tiger/tiger combination probably would be much easier logistically. Extinct rodent species likely would be the most appropriate model to begin with. De-extinction of species such as the dodo or carrier pigeon likely would be even more difficult than with mammals due to few developed tools for assisted reproduction in birds.

**Approaches to Recreating Extinct Species**

1. Modifying an existing species

With intense selection, one can markedly change the characteristics of a species in as few as 10 generations. There are current reverse selection efforts along these lines to generate aurochs from European cattle (17) One conceivably could evolve tigers to sabre-tooth tigers in this way or Indian elephants to mammoths. It might even be possible to accelerate this process by making modifications to DNA from information of snippets of DNA obtained from bones and other body parts of extinct species (2) While DNA from extinct species is usually highly degraded, a whole field of scientific activity has developed that involves determining essentially complete DNA sequences from body parts of people and other life forms that existed hundreds to tens of thousands of years ago. One example is the Neanderthal genome (15), with the surprising finding that those of us descending from Europeans still have a few percent of our DNA derived from interbreeding of our species with Neanderthals.

2. Breeding up if sperm are available

If reasonably intact epididymal sperm are found in a carcass frozen in permafrost, it may be possible to microinject such sperm into an oocyte of a closely related species to get a hybrid. For example, sabre tooth tigers and modern tigers may be more closely related than, for example, donkeys and horses, or lions and tigers. The cross could be fertile, as ligers are, or sterile like mules, although there are rare, fertile, female mules (1)

The sperm would be dead, but there are numerous examples of obtaining live offspring by intracytoplasmic sperm injection (ICSI) of dead sperm (e.g. 7) and freeze-dried sperm (8), in some cases stored at room temperature for 2 years (11). There is a potential problem relating to sex; for example, with bison X cattle crosses, the F-1 males are sterile and the females are sub-fertile.

Presuming that one could obtain a half sabre tooth tiger or wooly rhinoceros, etc., then one could repeat the process and get a ¾ version of the extinct species. Fortunately, such males (and females) likely would be fertile, as is the case for ¾ male beefalo. However, they would be highly inbred if sperm for the first and second crosses would be from the same individual. This would not be a problem if sperm were available from 2 or more unrelated, extinct males. Of course, even highly inbred animals often survive and do fine, but they are less robust than outbred ones. This issue might be somewhat compensated for by hybrid vigor from crossing species such as occurs with resiliency of mules.

3. Producing offspring by using 2 sperm

Decades ago, I proposed producing offspring with 2 genetic fathers and no genetic mother by removing the maternal chromosomes from an oocyte and fertilizing with 2 sperm (the mitochondria would still come from the oocyte as they almost always are inherited maternally.) Sometimes 2 sperm fertilize an oocyte simultaneously, so the blocks to polyspermy do not have a chance to inhibit one or the other. This particularly can occur with in vitro fertilization because many sperm are placed close to oocytes, a different dynamic than usually occurs with in vivo fertilization since few sperm are at the site of fertilization initially. When dispermy occurs, this triploid condition can be corrected at the I-cell pronuclear stage by simply aspirating one of the male pronuclei with a micro pipette. This occasionally is done with IVF programs. My "brilliant" idea was to create such embryos deliberately, e.g. by ICSI, and remove the female pronucleus. Without sexed semen, one would get a female ¼ of the time (2 X-sperm), a male ½ of the time (1 X and 1 Y-sperm) and a lethal embryo (2 Y-sperm) ¼ of the time.

Such a scheme would be great for genetic progress if using sexed semen because you could cross 2 males; before our current methods of genomic selection, bulls often had much more accurate genetic values than females. One could even cross a male with himself and get a female, although highly inbred! The female version would be to fertilize an oocyte with another oocyte instead of a sperm, or more simply to "fertilize" with the second polar body by suppressing its extrusion with transient incubation with cytocholasin B, one method of reliably inducing diploid parthenogenesis. Micromanipulations of polar bodies or pronuclei allows gynogenesis, the crossing 2 females.

What went wrong with the 2 fathers no mother or 2 mothers, no father scheme is the phenomenon that we now know as genomic imprinting, which means key cytosine base pairs (the C in DNA bases AGTC) are differentially methylated (C-Methyl) when the exact same DNA sequence is inherited via a sperm or an oocyte. These differentially methylated genes are primarily autosomal and complimentary, such that both sexes are needed to make viable embryos in mammals (13) If it were not for this requirement for complementary imprinting, women could reproduce without men (but with a fair bit of micromanipulation of embryos)!

Thus, my scheme of recreating extinct species in one step using 2 sperm met a huge roadblock due to imprinting issues, even though both sexes could have been produced, although highly inbred if both sperm were from the same male, and lethal in most cases due to deleterious recessive alleles.

In the last few years, female mice have been produced via parthenogenesis of inbred strains using complex and impractical transgenic modifications to the embryo (10), and 2 father no mother (except for mitochondria) inbred mice have also been produced by exceedingly complex procedures by having the male component pass through an ovarian stage to get the female imprint (4). de Boer and de Vries (3) have discussed various issues concerning application of two-father technology.

4. Approaches using genetic engineering of DNA

As indicated earlier, a whole field of endeavor has developed recently consisting of stitching together snippets of degraded, archaic DNA to obtain DNA sequences of essentially complete archaic genomes. Thus, it is likely that the complete DNA sequence of an extinct species can be assembled in the near future at a reasonable cost. The haploid genome of typical mammals has about 3 billion base pairs of DNA. It is one thing to determine such a DNA sequence, but quite another to synthesize it, although obtaining reasonably intact sperm would greatly simplify things.

In any case, if one had the appropriate DNA sequence and allelic information for the diploid state for an extinct species, it may be feasible to modify the genome of a closely related species to make an elephant embryo into a mammoth embryo, for example (2) With DNA sequences and cell line manipulations, and transgenic and cloning procedures (e.g. 18), this could lead to producing a fairly respectable mammoth embryo with elephant mitochondria, although even mitochondria could be modified to the mammoth version. Note that this sort of modification has already been done with the so called sleeping beauty gene (9), a DNA sequence that exists in mammalian DNA, but has been non-functional for millennia; with some tweaking, this gene has been brought back to functionality so that it is transcribed to make a functional transposon. For the extinct genome, changes to a closely related genome from a currently living species might be done in steps to make an intermediate species first, which already is done daily in simplified form to make genetically modified organisms (e.g. 18).

However, one likely would have to make hundreds of modifications to change a tiger genome to a sabre-tooth tiger genome instead of the few changes typically made for genetically modified organisms. There also would be the complexities of having appropriate allelic variations and the correct methylation state.

**Overview**

From the above examples, it likely will be possible to recreate some extinct species. I have emphasized mammals, which will be orders of magnitude more difficult than recreating extinct microorganisms or bringing back extinct yeasts, plants, insects, worms, etc. To recreate mammals would be excessively expensive, and in my opinion an inappropriate use of resources that currently could be better applied to problems of disease, contraception, etc. Nonetheless, tools for recreating extinct species are already available, and likely could be used successfully for that purpose, at least for simpler life forms, if sufficient investments were made. As indicated earlier, thinking about such projects stimulates other thinking that can be applied to current important endeavors. The teaching and learning benefits of these conjectures can be huge.

References


1. Allen, W.R., Short, R.V. 1997. Interspecific and extraspecific pregnancies in equids: anything goes. J. Hered 88:384-392.
2. Church, G. 2013. Reviving mammoths and other extinct species is a good idea. Sci Am 309, 12. Doi: 10.1038/scientificamerican0913-12.
3. de Boer, P., de Vries, M. 2011. Are there benefits from having two genetic fathers? Biol Reprod 84:409-411.
4. Deng, J.M., Satoh, K., Wang, H., et al. 2011. Generation of viable male and female mice from two fathers Biol Reprod 84:613-618.
5. Fehilly, C.B., S.M. Willadsen and E.M. Tucker. 1984. Interspecific chimaerism between sheep and goat. Nature 307:634-636.
6. Glenister, P.H., Whittingham, D.G., Lyon, M.F. 1984. Further studies on the effect of radiation during the storage of frozen 8-cell mouse embryos at -196° C. J. Reprod. Fertil 70:229-234.
7. Goto, K., Kinoshita, A., Takuma, Y., Ogawa, K., 1990. Fertilization of bovine oocytes by injection of immobilized, killed spermatozoa. Vet Rec 127:517-520.
8. Hochi, S., Watanabe, K., Kato, M., Hirabayashi, M. 2008. Live rats resulting from injection of oocytes with spermatozoa freeze-dried and stored for one year. Molec Reprod Dev 75:890-894.
9. Ivics, Z., Kaufman, C.D., Zayed, H. et al. 2004. The sleeping Beauty transposable element: evolution, regulation and genetic applications. Curr Issues Mol Biol 6:43-55.
10. Kono, T., Obata, Y., Wu, Q., et al. 2004. Birth of parthenogenetic mice that can develop to adulthood. Nature 428:860-864.
11. Liu, J., Lee, G.Y., Lawitts, J.A. et al. 2014. Live pups from evaporatively dried mouse sperm stored at ambient temperature for up to 2 years. PLOS One 9(6):e99809. doi:10.1371/journal.pone.0099809.
12. Lozhkin, A.V., Anderson, P.M. 2016. About the age and habitat of the Kirgilyakh mammoth (Dima): Western Beringia. Quarternary Sci Rev 145:104-116.
13. McGrath, J., Solter, D. 1984. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 37:179-183.
14. Menezo, Y., B. Dale and M. Cohen. 2010. DNA damage and repair in human oocytes and embryos. Zygote 18:357-365.
15. Prufer, K., Racimo, F., Patterson, N. et al. 2014. The complete genome sequence of a Neanderthal from the Altai Mountains. Nature 505:43-49.
16. Shapiro, B. 2015. Mammoth 2.0: will genome engineering resurrect extinct species? Genome Biol 16:228 DOI 10.1186/s13059-015-0800-4
17. Sinding, M.-H., Gilbert, M.T.. 2016. The draft genome of extinct European aurochs and its implications for de-extinction. Open Quarternary 2:7. Http://doi.org/10.5334/oq.25
18. Whitworth, K.M., Lee, K., Benne, J.A. etal. 2014. Use of CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. Biol Reprod 91:78, 1-13.