To clone a dinosaur

Quaggas and mammoths are extinct—but genes and proteins extracted from their mortal remains can 'live' again

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CAN extinct animals be brought back to life? Palaeontologists might say "yes", but they would mean only that they can reconstruct the bones and muscles and work out how the animal walked and what it ate. Yet recent research suggests that we may eventually be able to clone an extinct animal.

Palaeontologists have long known that organic material can survive in fossilised bones. In 1908, a Dr Bennett of the Natural History Museum in London reported organic tissue attached to the teeth of some fossil fishes. In the early 1960s, several scientists in Britain and the US began to study the proteins of fossil bone in more detail. Ralph Wyckoff and colleagues of the University of Arizona found the protein collagen in fossil bone of the Pleistocene (about 1 million years ago; MYA) and the Miocene (5-24 MYA). The crucial evidence came from an examination of ultra-thin sections of the bones and teeth of a variety of mammals under the electron microscope. Collagen, a fibrous substance that forms a tough and flexible mat within bones, teeth and tendons, is characterised by a regular pattern of banding. Each fibre is divided into light and dark stripes which alternate every 64 nanometres. In the fossil material, Wyckoff found the same banding, although the spacing was reduced to about 60 nanometres in many Pleistocene specimens, and to about 50 nanometres in the Miocene fossils. Further, when samples of these fossil bones and teeth were analysed chemically, the proportions of various amino acids making up the protein turned out to be very similar to those of the collagen in modern bone.

The African quagga was hunted to extinction in 1883. But we have extracted its DNA from a salt-preserved skin

Collagen has also been identified in older fossils, ranging from fish, 190 MY old of Lower Jurassic age, to graptolites, 500 MY old of Ordovician age. Graptolites were colonial, floating organisms that are known only from their thin external skeletons—often shaped just like hacksaw blades—which are abundant in ancient dark mudstones. Under the electron microscope, the graptolite's skeleton has a regularly banded, fibrous appearance which mimics the structure of present-day collagen. However, chemical analysis has failed to detect any sign of amino acids in the graptolites. An impression, or ghost, of the detailed structure of the skeleton is all that remains, and all traces of the original organic material have been lost.

This example points to one of the main problems in studying the proteins of fossil vertebrates: the vicissitudes of preservation. In general, the processes of fossilisation and the length of time that a fossil remains buried significantly affect both the protein content and its amino-acid composition. A fresh bone contains about 20 per cent protein by weight, whereas a fossil bone only 1 MY old may have only 0·01 per cent or less protein. Extensive studies in the 1960s of bones and teeth from the Pleistocene Rancho La Brea Tar Pits of Los Angeles showed that their amino-acid compositions were similar to those of modern collagen. However, older fossils had quite different collagen, and it is clearly important to try to assess how much of these variations actually reflect the original collagen, and how much has been produced by the processes of fossilisation and burial.

Some of the amino acids in bone also occur quite frequently in sediments. Thus, a serious criticism of the chemical analyses of fossil bones could be that all of the amino acids came from the enclosing sediments, and that none, or very few of them, actually mirrored the original collagen. So we need to measure the amino acid composition of both the bone and its enclosing sediment as a control.

In a recent review of the fossil proteins in bones, W. G. Armstrong and his colleagues at the Royal Dental Hospital of London School of Dental Surgery found little evidence for intact collagen in any fossils older than the Pleistocene. In most cases, the fossils contained contemporary proteins, but these proteins were most likely derived from bacterial contamination. In the oldest samples, 400 MY old, sediment and bone were very similar: the amino acids of the original collagen, the decomposing bacteria, and the sediment had become completely mixed.

The conclusion is, then, that collagen does not remain intact in fossil bones for as long as might have been hoped. We can expect to extract this protein with any confidence only from Pleistocene fossils, although in rare cases small amounts may survive from earlier times, say up to 150 MYA at most. There is still some hope of extracting dinosaur proteins!

The proteins of fossil vertebrates have already helped researchers to assess the relationships of extinct animals in two ways: by studying immunological reactions and by discovering the sequence of bases making up cloned DNA.

In 1980 Jerold Lowenstein, of the University of California in San Francisco, developed a radioimmunoassay technique for extracting and measuring tiny quantities of protein from the bones of extinct animals. The bones, teeth or skin are ground to a fine powder, and the collagen is extracted in acetic acid. The technique then depends on simple immunological reactions: a species recognises proteins from another species as "foreign" and develops specific antibodies against them. Extracts of collagen from fossil bones and from the bones of related living species are injected into rabbits to produce antibodies specific to each collagen solution. The various antisera (antibodies in solution) are then added to the original collagen solutions, and they "recognise" and bind to their "own" collagen. Antibodies derived from the collagen of the same species show most binding, and those from distantly

related species show less binding. The amount of binding is measured by attaching a second, radioactively labelled antibody to the first set of unlabelled antibodies. The amount of radioactivity in the sample then reveals the degree of binding.

Lowenstein has now studied the immunological relationships of several extinct animals. In 1981 he examined some human fossils ranging from an Egyptian mummy 3000 years old to a specimen of *Australopithecus* (1·9 MY old). These all yielded collagen that could be tested immunologically with various living apes and with humans. The results confirmed other biochemical data that apes and humans were much more closely related, and that they shared a much more recent common ancestor than had previously been believed from the fossil evidence—5-10 MY, rather than 15-30 MY (*New Scientist*, 3 May 1984, p 24).

Lowenstein and his colleagues also tackled the Tasmanian wolf (*Thylacinus cynocephalus*), an Australian animal which looked like a wolf, but which was, of course, a marsupial, a pouched relative of the kangaroo and the opossum. The last Tasmanian wolf died about 1930, although there are reports of more recent sightings. The animal's relationships are disputed: was it related directly to the Australian dasyurid marsupials, or to the extinct dog-like South American marsupial *Borhyaena*? Lowenstein obtained some muscle and skin from a stuffed specimen in a museum and tested its immunological relationships. He settled the argument in favour of the dasyurids.

More recently, Lowenstein's group has examined the relationships of the extinct Steller's sea cow (*Hydrodamalis gigas*). This gigantic herbivorous marine mammal was first observed in 1741 on the Kamchatka Peninsula in eastern Siberia. By 1768 it had been exterminated. Bone from surviving skeletons of Steller's sea cow was compared immunologically with various living mammals, and the closest resemblance was found with the Indo-Pacific dugong, and with the American and African manatees.

Palaeontological estimates had put the time of the sea cow's separation from the dugong at about 20-30 MY, whereas the immunological data suggest something more like 4-8 MY. Further, the palaeontological estimate for the dugong-manatee split was about 45 MY, but the new immunological data point to a common ancestor that lived only 17-20 MYA.

This molecular study, like many others, has tended to shift common ancestors upwards in time—nearer to the present day. Palaeontologists, relying on interpretations of fossils to place dates on times of divergence, have tended to get carried away with "ancestor-hunting". Thus, in the human example mentioned above, the sticking point in reconciling the palaeontological and molecular dates of divergence of the apes and humans was a fossil called *Ramapithecus*, dated at 8-14 MY. This fossil was regarded as an early ancestral hominid, and it was certain then that humans and apes must have diverged before 14 MYA. It now seems that *Ramapithecus* does not have any particular human characters, and it can easily be accommodated as an early form that was neither human nor ape, or even as somewhere near the orang-utan line. The sea cow may provide another example of the dangers of ancester-hunting. Palaeontologists had placed the manatee-dugong split back in the Eocene (about 45 MYA) because an early animal, *Eotheroides*, has been identified as a dugong. A re-examination of the specimens may show that *Eotheroides* is just a generalised sea cow, with no particular relationship to either dugongs or manatees. We must beware of the *Guinness Book of Records* approach to fossils—the desire that is, to find the oldest or the first example of a particular living group without due consideration of the characters of that group. Does the fossil really have one or more of the diagnostic features of the living group, or is it merely a plausible ancestor with no such features? We must be suspicious of "plausible ancestors".

The second, and more difficult, approach to using the proteins of extinct animals for assessing their relationships lies in sequencing their DNA. For the first time last year, Russell Higuchi, Allan Wilson and their colleagues at the

Researchers can harvest collagen, a protein in bone and skin, from human fossils—ranging from an Egyptian mummy who died 3000 years ago (above) to the remains of an Australopithecus *(left) nearly two million years old. A young mammoth, preserved in the Russian permafrost, (above right) is another source of "extinct" protein. Biochemical analyses can reveal how closely such creatures are related to living animals*

University of California, Berkeley, succeeded in extracting, cloning and sequencing the DNA of the quagga (see *New Scientist*, 13 December 1984, p 21).

The quagga (*Equus quagga*) was a zebra-like animal that lived in Africa but was hunted to extinction by 1883. Higuchi and Wilson obtained a small piece of dried muscle and connective tissue from the salt-preserved skin of a quagga that died 140 years ago. They extracted DNA, but harvested only about 1 per cent of the amount normally present in fresh muscle. They tested this DNA for microbial contamination by comparing it with the DNA of the living mountain zebra and then cloned the DNA in bacteria to increase the amount available for analysis. The pieces of DNA gave a strip 229 bases long, which the researchers compared with the DNA of the mountain zebra. There were differences at only 12 bases, and only two of these differences would have produced different amino acids in the protein.

Such small difference in the sequences (about 5 per cent of the bases) would be expected between any two closely related species. It is similar to the amount of differentation between the other living species of *Equus*, the horses and zebras. Thus, the quagga is not a particularly divergent form, and the molecular evidence suggests a date of 3-4 MY for the split between the mountain zebra and the quagga.

This date was obtained by calibrating the "molecular clock" against dates for the splitting of major lineages drawn from the fossil record. The molecular clock hypothesis is that DNA, and proteins, show approximately regular rates of substitution in their bases, or amino acids, over time, and that a measure of molecular difference gives a measure of phylogenetic distance (particularly, the age of the latest common ancestor between two or more forms). In this example, the fixed date is for the divergence between cow and human at 80 MYA, and this corresponds to a corrected value of 230 base substitutions between the two. The 12 base differences between quagga and mountain zebra correspond to about 5 per cent of 230, thus 5 per cent of 80 MY, which is 3-4 MY.

DNA has now been extracted in some quantities from the flesh of mammoths, frozen in the permafrost of Siberia 40 000 years ago. However, most of the DNA in these cases comes from recent bacterial contamination, and elephant-like DNA is present in only tiny quantities, and much of this is degraded. Cloning enough mammoth DNA for sequencing would be a huge task, and even then it would be difficult to work out how much the DNA had altered as a result of burial and fossilisation.

Will we ever be able to clone a dinosaur? We can obtain collagen from Pleistocene animals (that is, up to 2 MY old), and even from many earlier forms from the Tertiary (2-65 MYA). Amino acids can occur in older fossils, but it becomes harder to disentangle the original vertebrate collagen from contamination by bacteria and sediment the further back in time one goes. Nevertheless, small quantities of original collagen probably exist in fossils of Mesozoic age (65-245 MYA), including dinosaurs.

It may become possible to concentrate these tiny quantities of collagen, by grinding up huge quantities of fossil bones, or even to stimulate the multiplication of the collagen fibres on an appropriate nutrient medium. Then the radio-immunoassay technique of Lowenstein could test the immunological relationships of animals belonging to wholly extinct groups, with each other, and with living forms.

Much more speculative is the idea that the DNA of extinct organisms could be cloned to reconstruct all of its genetic materials. The DNA from, say, a quagga, could then be inserted into an early embryo of the mountain zebra, in the hopes that it would take over the processes of development. Researchers could take a zebra's embryo that had just been fertilised, remove its nucleus and replace it with the quagga's DNA. The chances of success seem remote at present, so we have a long way to go before biologists can produce a cloned dinosaur. For a start, they would have to get some dinosaur DNA, and no-one has found any of that yet; then they would have to clone it. And what on earth would you choose for the surrogate mother of a dinosaur? □

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Steller's sea cow: discovered in 1741, extinct by 1768