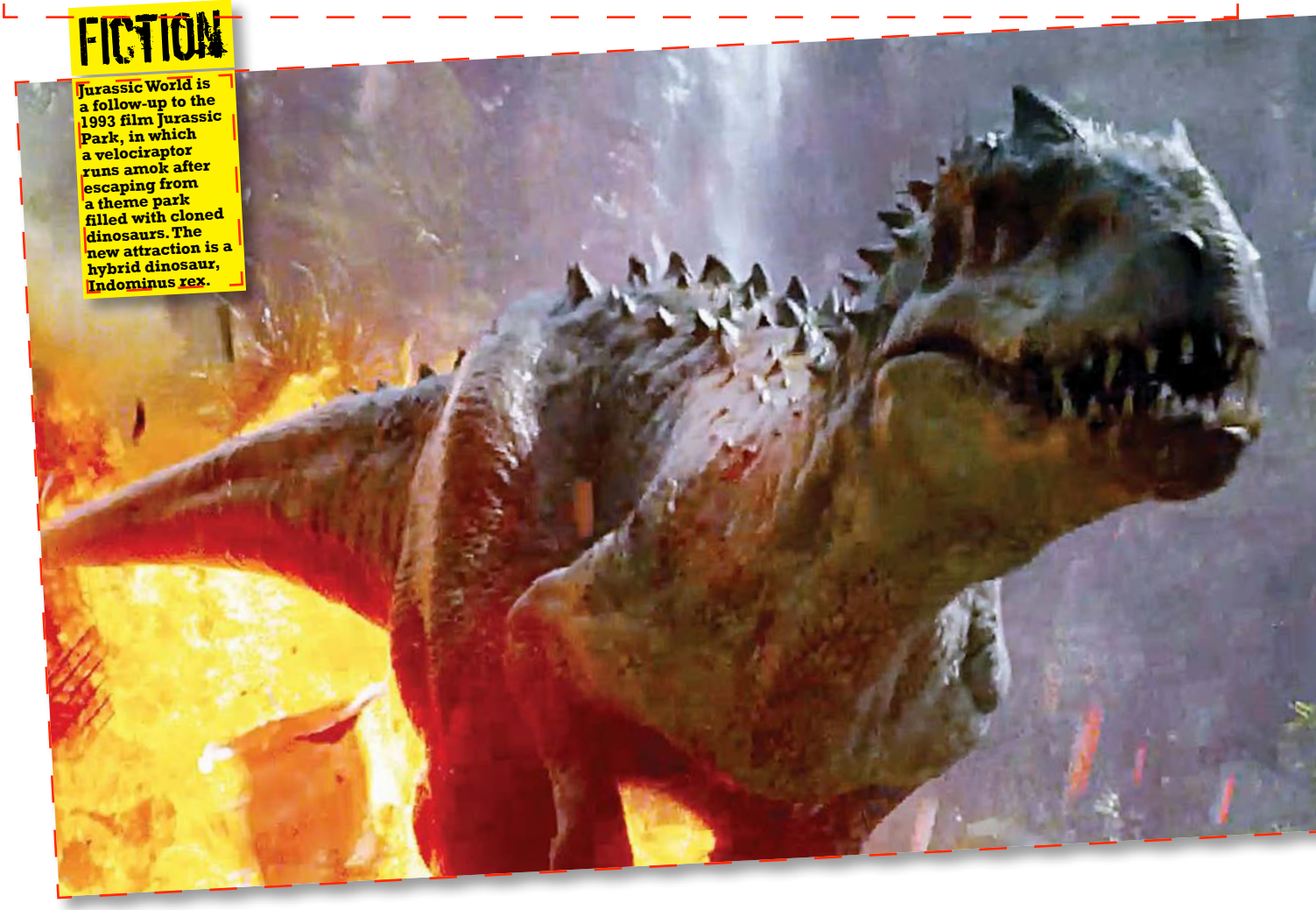
Genetic engineers are working to restore extinct species to life. But doing so involves solving a massive jigsaw puzzle with missing pieces. By **Chris Edwards**

Recipe for de-extinction



FICTION

Jurassic World is a follow-up to the 1993 film Jurassic Park, in which a velociraptor runs amok after escaping from a theme park filled with cloned dinosaurs. The new attraction is a hybrid dinosaur, Indominus rex.

WHEN HE VISITED the George Poinar's lab in the 1980s, author Michael Crichton saw a way to lend plausibility to the plot of his novel-in-progress 'Jurassic Park'. Poinar was working to discover whether amber's ability to preserve the tissues of insects and other tiny creatures could also protect the DNA locked away inside the cells of those tissues.

In a case of life imitating art, Poinar gave samples of amber to his son Hendrik who teamed up with Raúl Cano of California Polytechnic. Cano had developed and subsequently patented a process for extracting DNA from organisms trapped in the yellow resin. They extracted fragments of DNA from an ancient, stingless bee and then more extensive samples from a weevil that lived 120 million years ago – almost twice as long ago as the cataclysm that wiped out dinosaur species such as Tyrannosaurus rex.

The paper on the recovery of DNA from the weevil appeared in the journal *Nature* a day after the movie's premiere in Washington DC. It seemed that the idea of bringing long-extinct species back to life might be possible, even if they were only insects.

However, attempts to reproduce the original results failed and realisation dawned that the amber samples were hopelessly contaminated – much of it from the DNA of modern bacteria that had either colonised the samples, or had been picked up in the lab itself. The idea of being able to sequence the DNA of a mosquito trapped in amber millions of years ago, let alone any dinosaur DNA rapidly faded.

Ice-age revival?

But the more stringent lab protocols developed by scientists such as Svante Pääbo – together with increasingly sophisticated extraction techniques – have yielded usable ancient DNA from other long-extinct species, including our own Neanderthal relatives.

Ice-age species such as these and mammoths show prospects as good as any for revival. It's not just the relative youth of the fossils that helps. Some people want to go ahead and bring them back in the hope of reviving lost ecosystems.

Stewart Brand, president of The Long Now Foundation and the architect of the group's 'Revive and Restore' project, says: "Because it's cold in the Arctic and sub-Arctic, the DNA is relatively intact."

FACT

Velociraptors were little bigger than turkeys, and recent fossil finds indicate that they probably had feathers too. But there's no prospect of recovering enough sound DNA to clone them.

But ice-age DNA is in far worse condition than any DNA that can be extracted from the living. And all but the most recently extinct species yield DNA that is not in the form of whole chromosomes millions of base pairs long. Beth Shapiro, scientist and author of a recently published book on the practicality of de-extinction, describes it as "more like confetti that's been run over by mammoths in the rain".

Pääbo says of the experiments conducted on Neanderthal samples: "The DNA fragments are very, very short. Just 50 or 60 bases. And even the best bones result in just 3 or 4 per cent Neanderthal DNA. The rest are from bacteria that colonise the bones."

Even the constituent bases that make up DNA go through chemical changes as the polymer degrades. Cytosine – the 'C' in the familiar four letter 'GTCA' genetic code – is often changed to uracil. In turn, this leads to errors in sequences, because the biological agents that generate multiple copies of a DNA strand to provide sequencing machines with more raw data miscode uracil, inserting a T for thymine rather than a C. Other chemical changes stop the enzyme that creates DNA copies in its tracks, yielding even less information from the highly fragmented chains.

Chemical error correction has made it possible to recover from many of these problems. But there are still problems for de-extinction proponents. Describing the process used to construct what is, for the moment, the best sequence for Neanderthals, Pääbo says: "We sequenced a little over a billion fragments from the DNA recovered from the bones. Parts were missing but in the end we had a little over half the total genome."

Not only is a large portion of the DNA missing, there is no way to determine from the DNA itself how it should be structured in a reconstituted genome. Bioinformatics, biology's IT-oriented arm, can deal with some of the problems. Because individual strands of DNA will break down in different ways, there are often overlapping segments. By comparing these segments, computer software can piece together large parts of the molecular jigsaw puzzle of ancient DNA. By comparing the DNA to the genomes of modern species, it often determines whether they are contaminants. But some parts of >

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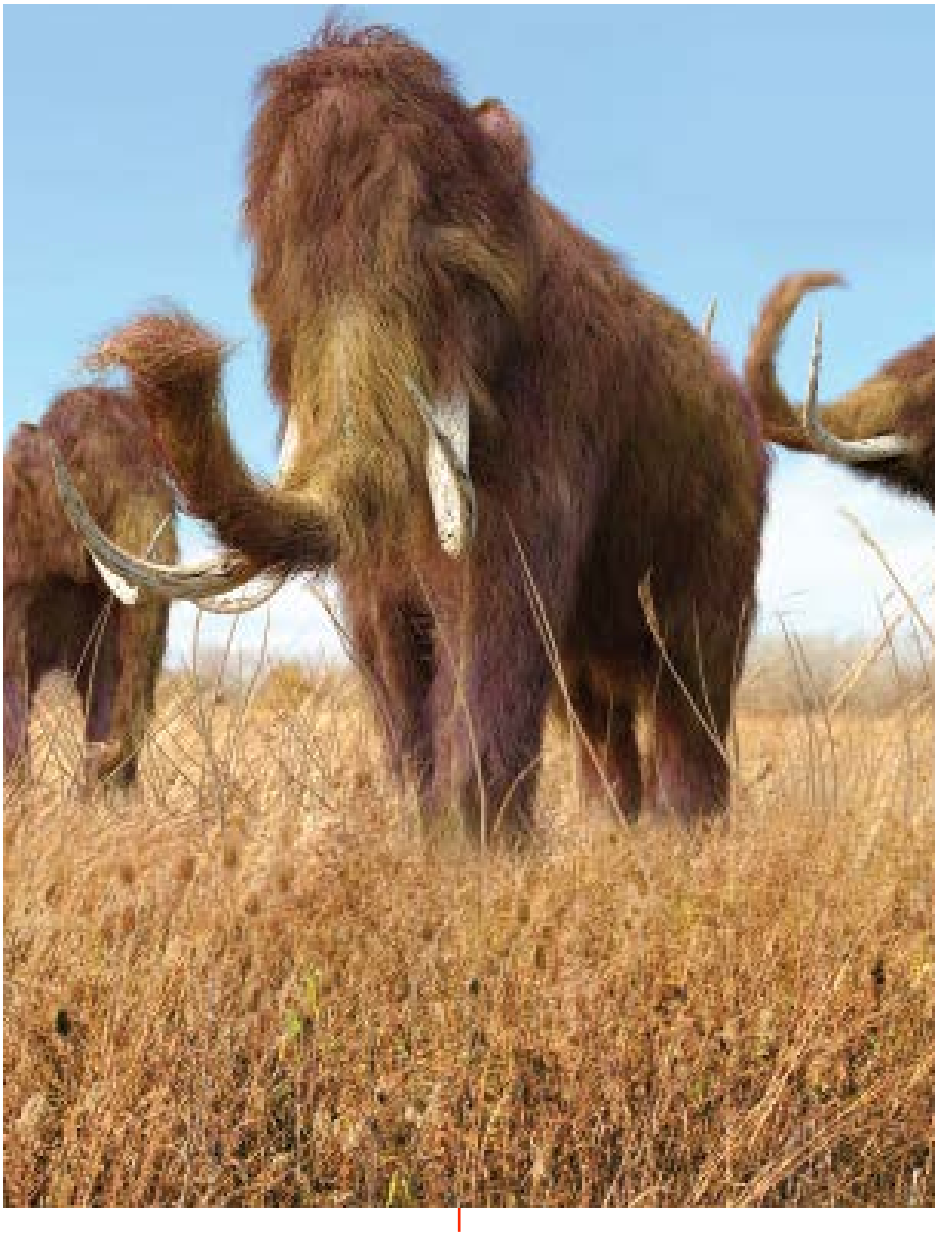

< the genome are currently beyond our reach for another reason.

Shapiro says: "How many genomes have been completely sequenced? None. Not even our own genome, for which a ridiculous amount has been published."

Missing links

Potentially important missing components are lengths of DNA called heterochromatin. The problem for sequencing heterochromatin is that it is highly repetitive and the repeating sequences are very short. There is no practical way for software, using the data that comes from sequencing machines, to determine how many repetitions there should be because overlaps produce little useful additional information.

The role of heterochromatin is fuzzy, but it seems to have a role in regulating when and where genes are active. As regulation of genes is a key factor in the developing embryo, subtle changes to heterochromatin could result in massive modifications to the organism. Without more information on heterochromatin's role, it is impossible to determine what ancient DNA sequences should even begin to look like. But this need not stop the de-extinction process.

Crichton's other plot element used both for biological plausibility and to deliver the novel's warning – weaving together genes from today's species with those from ancient DNA – may provide the template for de-extinctions in the short to medium term. It is likely to make more sense to start with modern DNA and add in the elements needed to reproduce the ancient species, as the heterochromatin and other poorly understood parts of the genome are already in place. Based on the genomes performed so far, the Asian elephant seems to be a closer relative to the mammoth than the African elephant. This has given geneticists hope that relatively simple changes could result in a surrogate mother elephant giving birth to a baby mammoth. "We could have the first mammoth embryo in three years' time," Brand claims.

George Church's group at Harvard University in Boston, which appears to be in the lead in manipulating elephant DNA to be more mammoth-like, has yet to demonstrate in published research that the genes stitched into elephant eggs result in the mammoth DNA being used even in early development.

But Church's team has developed efficient techniques for editing DNA. Conventional genetic engineering by comparison is hit-and-miss. Church's CRISPR technique makes it possible to insert DNA in a specific part of a chromosome in such a way that the organism is unlikely to reject the addition.

Reverse-engineering DNA to find out how it works is likely to be the only way that dinosaurs – or at least animals that resemble them – could possibly be brought back to life. There are other ways to recover lost DNA information than to sequence the fragments recovered from ancient specimens. A multi-university team realised their chances of finding DNA from a group of species that lived in ice-age South America were remote.

CRISPR GENE DELIVERY

MAMMOTH BID FOR ECOLOGICAL RENEWAL

For Sergey Zimov, head of the North-East Scientific Station in Russia, bringing the mammoths back to the tundra is not a matter of satisfying curiosity about how the animals would look in real life. For about a decade Zimov has led the development of 'Pleistocene Park' in northern Siberia – an attempt to recreate an ice-age ecosystem and, in doing so, tackle the problem of greenhouse gas emissions as the modern tundra warms.

With varying degrees of success, Zimov's team has reintroduced larger animal species to the area in the hope that their presence will alter the landscape. By eating and trampling the vegetation, the animals would restore ancient grasslands, which act more as a carbon sink than a massive reservoir of methane produced by the decay that prevails today. Bison have failed to take hold but reengineered mammoths adapted to the conditions of northern Siberia could be far more efficient at keeping the grasslands under control, assuming Zimov's theory about the Pleistocene ecosystem holds.

According to Harvard University researcher, George Church, the techniques used to engineer mammoth characteristics, such as cold-adapted haemoglobin and thicker fat layers under the skin are likely to be medically useful. The CRISPR gene delivery technique used at Church's lab to insert mammoth genes into elephant genomes has potential for reinvigorating gene therapy and by adding greater variations to species endangered by a lack of genetic diversity, such as the Tasmanian Devil.

Former methods for adding foreign genes resulted in them being inserted at random points in the chromosome, sometimes triggering cancers. The CRISPR approach targets specific DNA sequences, allowing the gene to be attached at a precise point in the genome.

However, there are risks to CRISPR-like techniques. Church criticised the publication of a paper earlier this year on an approach that could be used to spread a mutation through a species. Although there are uses that are benign to humans – such as altering mosquitos to no longer carry malaria – a lack of safeguards in the

Although a group that included Hendrik Poinar managed to extract DNA from ice-age rodents found in Mexico, the region where these ungulates lived was too warm and wet for even well-preserved specimens to not have their DNA destroyed. But proteins that make up hair and other parts of the body are much more robust. Enzymes insert each amino acid in a protein in the order determined by the DNA sequence of the corresponding gene. Find variations in the proteins and you find how those genes differ from those used by other species. This is also one of the approaches being used by Mary Schweitzer of North Carolina State University, who found remnants of soft tissues and collagen in dinosaur bones. Although the sequences show similarity to bird proteins, they may yet be found to be modern contaminants.

There is another approach. Work has started on uncovering the genetic information that birds discarded over the past 65 million years by, in effect, rewinding their genetic clock. Scientists already think dinosaurs were a lot closer to birds than the giant lizards of popular imagination. In the movies, velociraptors are scaly. A number of fossils have turned up since that give clear indications that the animals were feathered and may even have been able to glide over very short distances.

Turn back the clock

The search for a way back has already begun: with hen's teeth. In 2006, scientists from the University of Wisconsin and the University of Manchester succeeded in coaxing the embryos of chickens to grow teeth similar to those found in dinosaur fossils by reactivating latent genes. Other work, based on the intuition that bird embryos briefly grow lizard-like tails before further development causes them to develop as those of birds, points to a number of individual genes lingering from the days of the dinosaurs that may have mutated very little over millennia because they are still required by the growing organism. Similar work by Richard Borowsky at New York University has given eyes back to blind cavefish, a species that has lost the ability to see since the Pleistocene.

Mutations that render the genes entirely inactive would put the animal itself at risk. What has changed is the framework that puts them to use. By working out ways in which these genes can be reactivated and develop more dinosaur-like features, scientists such as Jack Horner – who acted as consultant on the movie of Crichton's novel – believe it might be possible to reconstruct the creatures by engineering modern bird DNA.

But it's far from clear how much new DNA needs to be synthesised in order to recreate features that have been mutated out completely over millions of years. DNA that is not needed by species quickly turns to junk or is co-opted by other mechanisms in the cell if some of the mutations turn out to be beneficial. Simply rewinding the genetic clock and generating dinosaurs by atavistic mutation of chickens to create what might be called an 'alektosaur' is not a realistic option. De-extinction is likely to need new DNA to be created.

Brand says: "I think it's fair to say this century will be largely defined by intersection between the kind of coding [IT programmers] do and the kind of coding that life does."

But even if radical genetic engineering succeeds in constructing something that looks like a dinosaur, it is clear that it can never bring back what existed more than 65 million years ago. The specific information in those genomes has been chemically shredded forever. Such a chimera would have a lot in common with the beast that underpins the plot of Jurassic World. Maybe life can imitate art.*

INCONVENIENT FACT

REBOOT PROBLEMS

In the popular imagination, J Craig Venter's laboratory created life from scratch five years ago by synthesising completely *de novo* DNA, including the monograms of key researchers in parts of the chain that would not be treated as genes. But the reality is, in order to start working, the genome had to be inserted into an existing bacterial cell that had all the apparatus in place to deal with the new DNA.

The overall effect was of 'rebooting' a cell rather than creating life from scratch. Without a host of existing enzymes ready to manipulate and work on it, the DNA would just be a white, sticky blob in a test tube. Even with the support infrastructure of a readymade cell, the bacterial environment that Venter's team dealt with is much simpler than in the animal cells that de-extinction researchers will deal with.

For Venter's team it was enough to simply insert new DNA. Plant and animal DNA needs an entire infrastructure around it to operate successfully and that infrastructure plays a major role in determining how the organism develops. Scientists have come to understand that reading the DNA sequence itself is not enough. Subtle chemical changes and even the way in which the DNA is wrapped around protecting lumps of protein help determine which genes are read by the cell's machinery and which are temporarily ignored.

How much those epigenetic alterations matter to determining whether a mammoth develops from what appears to be mammoth DNA is not well understood today.