ANCIENT BIOMOLECULES INITIATIVE

Molecular Signatures from the Past

Red light for amber DNA

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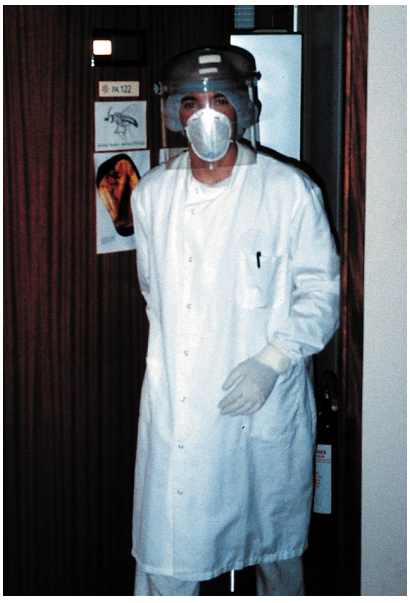
Trigona gribodoi, a stingless bee, preserved in East African plant resin (copal) - one of the specimens from which we attempted to recover ancient DNA.

Although DNA fragments have been successfully recovered from archaeological material less than 50,000 years old, claims that DNA survives over millions of years remain contentious. Special conditions are needed to preserve ancient biomolecules, and it is generally agreed that fossils trapped in plant resin (amber) offer the best chance of preserving DNA over geological time-scales. We found no evidence that DNA can be preserved in ancient amber.



Protective clothing is essential to minimise the problems of contamination when working with ancient DNA.

Despite the publicity given to reports of ancient DNA being recovered from dinosaur bones and eggs, 17-million-year-old plants and organisms trapped in amber, more careful scrutiny of the results has cast doubt on all but the amber work. Even for organisms in amber, the authenticity of the DNA sequences remains uncertain because the results have never been repeated in an independent laboratory.

We tried to recover ancient DNA from well-preserved insects entombed in Dominican amber (15-20 million years old). We concentrated on the species of bee from which ancient DNA was first reported, and used a variety of different methods to try and extract its DNA. Small pieces of DNA from regions of gene previously obtained from amber insects were targeted. We also tried to obtain DNA from smaller-bodied flies and from much younger insects preserved in East African plant resins. No genuine ancient DNA was recovered from any of our samples. Occasionally we obtained some DNA which appeared to be authentic, because the control experiments gave the expected negative results. Yet cloning and sequencing has shown that this DNA comes from modern contaminants, despite our efforts to avoid contamination.

Our inability to recover ancient DNA from insects trapped in fossil plant resins, the most promising of media for the preservation of DNA, implies that DNA is extremely unlikely to survive over geological time-scales.

The Ancient Biomolecules Initiative is a five-year programme to understand the fate of biological molecules in archaeological and fossil materials, and to explore the applications of this new knowledge.
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Natural Environment Research Council

Red light for amber DNA: the science in detail

Although DNA has been recovered from amber-entombed organisms, proving whether it is genuinely ancient in origin is not easy. DNA fragments recovered from fossil organisms ought to meet three criteria before being accepted as authentic.

- They must be obtained under strict procedures designed to minimise contamination from modern DNA
- The sequences must make phylogenetic sense, ie DNA from ancient bees must look more like modern bee DNA than like fungal DNA
- Results must be reproducible by other laboratories.

Methods

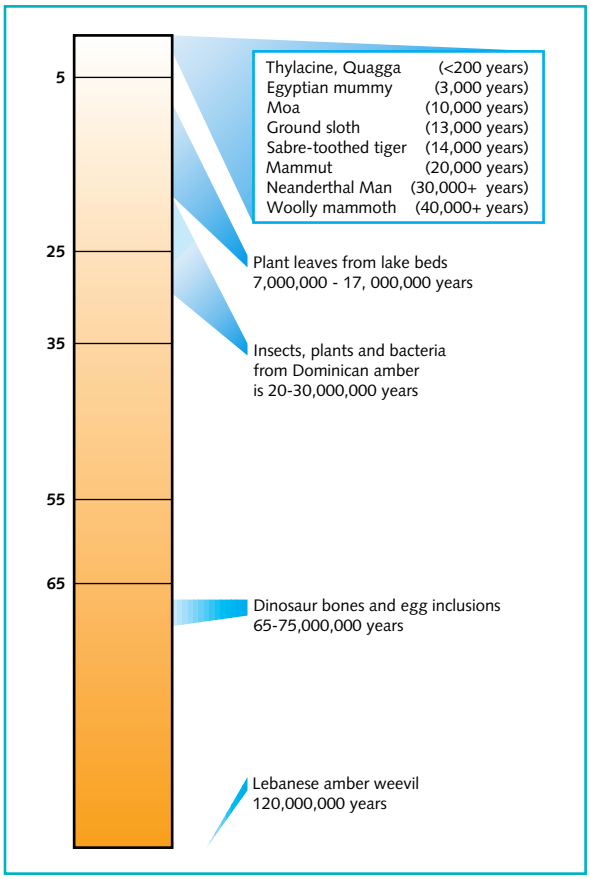
We have attempted to repeat the original work of Cano *et al*. on bees trapped in Dominican amber. To ensure that contamination from modern sources was minimised, we established a new laboratory dedicated to DNA extraction from amber insects and developed stringent laboratory procedures that far exceed the minimum recommendations for ancient DNA work. Fossil insects were carefully checked, and only those entirely encased in amber, free of cracks and imperfections and with dense mummified tissue, were used. Tissue was extracted from head, thorax and abdomen of ten specimens of *Proplebeia dominicana*, the bee that yielded the first supposed ancient DNA sequences. Tissue was also extracted from two specimens of much smaller-bodied flies (the phorids *Megaselia* and *Puliciphora*) from Dominican amber, and from three specimens of another bee, *Trigona gribodoi*, from East African copal (less than 100,000 years old). Five DNA extraction techniques were routinely used, all of which have been successfully employed on ancient material. We optimised the polymerase chain reaction (PCR), a method which can make many thousands of copies of a stretch of DNA starting from as few as 10 template molecules. We looked for two different genes in the insects, one nuclear and one mitochondrial, using combinations of 16 different primers.

Results

Most PCR attempts failed to yield any DNA from the insects in amber. However, seven (out of 156 attempts) did produce DNA of the expected size. Unfortunately, amplified DNA sequences were not reproducible when we repeated PCR on the same specimen, nor were the sequences consistent between amplifications. The sequence of the recovered DNA fragments showed them to be modern contaminants.

Our attempts using the same (and additional) DNA extraction and PCR amplification techniques, targeting the same (and smaller) DNA fragments from the conserved gene regions used in previous studies, have failed to recover authentic ancient insect DNA.

We have examined more specimens, covering a wider range of body sizes and fossil resins than any previous 'successful' study published. We have to conclude that previous studies have been misled either by minute amounts of contaminating DNA or by the vagaries of molecular biological techniques acting on extremely small quantities of damaged DNA. Although no negative result can disprove the existence of ancient DNA in amber-preserved fossils, our work shows that the isolation of geologically-ancient DNA cannot effectively be reproduced.



Above: Geological timescale (in millions of years) showing some of the more important reports of ancient DNA recovery.

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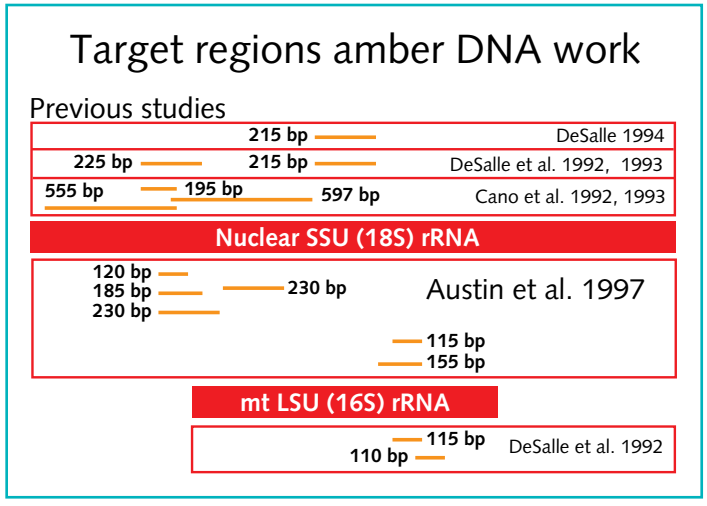
Below: Regions of mitochondrial and nuclear gene targeted by previous workers and by us.



Phylogenetic analysis shows that all the DNA fragments we amplified from fossil insects (in this case *Trigona*) are more likely to be contaminating fungal and vertebrate sequences than insect sequences.