RESEARCH

HOW ANCIENT DNA WAS ABLE TO IDENTIFY THE EXTINCT ROCK-WALLABY ON DEPUCH ISLAND

Peter Spencer

As many people would be aware, we have witnessed an appalling number of mammal extinctions since European settlement, accounting for about one-third of all extinctions worldwide. The most severely affected Australian mammals have been the marsupials. Because many are now extinct, there is nothing left to 'discover' about these unique animals and their only reminder of what they looked like and what they might have done are left in museums and collected as bones in places such as caves. One recent example of this was a rock wallaby (*Petrogale sp.*), a small kangaroo species from Depuch Island, off the Pilbara coast. This taxa only recently vanished (around the 1970s) and the main reason is probably because of predation by introduced foxes.

One of the problems with the Depuch Island rock-wallaby was that its taxonomic status remained unknown, that is, we don't know exactly which species it actually was. It could be one of two possible subspecies of *P. lateralis*, each possessing a different genetic 'signature'. There are a number of different genetic markers which contain well-defined regions for discriminating differences among marsupial taxa. Therefore, in order to determine the subspecies status of this rock wallaby we used DNA, the building blocks of life. DNA extracted from any sample can identify the species, gender, and provenance (location or place of origin) of the sample animal. It can also provide direct estimates of parentage (lineage) and even the genetic variations within a population. This 'fingerprinting' uses the same technology that human forensic science uses to solve crimes. DNA is the perfect method to determine the species because first, it does not rely on external appearance, and second DNA is a genetic time-capsule containing our history and preserving it long after death.

Ancient DNA is a general term used to describe any genetic sample from 80,000 years ago up to last week. Technically, finding human remains that are less than 50 years old is still considered to be part of a forensic investigation. However with animals, once it's dead, it's considered to be ancient. What is more exciting is that there are more than 1.5 billion samples of ancient DNA in museums around the world in the form of bones, skeletons, teeth and even old documents or clothing made from the hide of animals, many of whom are now extinct. The only drawback is that many would be contaminated by other DNA. For instance, just think about how many people may have handled a 100 year old bone in a museum. Everyone who handled it would have 'contaminated' the sample with their sweat, skin cells, hair and even by just breathing on it. What has to be remembered is that there is probably only a little bit of 'real' DNA left in the bone, and it doesn't make sense to get a result from the last person who handled the specimen! Distinguishing between the *real* and *contaminating* DNA is the important trick in ancient DNA analysis.

This is exactly what we did with the Depuch Island rock-wallaby. DNA was extracted from a tooth-root (which, because it is embedded in the lower jaw is less likely to have been contaminated) and a compact bone sample of the femur, in an ancient DNA laboratory in Germany. The amplified fragments could easily be seen on a special gel for looking at DNA. The result is a sequence of lines in a DNA pattern, rather like a bar code, which is unique to each species (see fig 1). Comparing the codes with each other enables the researcher to determine how closely related they are. This is the same technique that was used to identify endangered species of whale being sold as meat for human consumption in Japan, but with the added problem of going back in time. Sequencing these fragments allowed us to distinguish the species as *P. lateralis lateralis*, which is still found on mainland Australia, but not directly adjacent to Depuch Island (that species of rock-wallaby is the Rothschild's rock-wallaby, *P. rothschildi*).

A clear conservation outcome from this work was the demonstration that the tooth could

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P. sp. unknown*  AAATTCTTACTCATGTCAGTATCAAACCACCTACCGTACCTAG
P. l lateralis1  ........................................
P. l lateralis2  .......................................A
P. l. hacketti3  ........................................
P. l. pearsoni4  ........................................
P. penicillata5  ..........T.....................T........A
P. herberti6     ..........T.................C.TG.......A

P. sp. unknown   TTTATTTTGTTTTTTT-ATGCACGTACATATATATGTATAACT
P. l lateralis   ................T.........................
P. l lateralis   ........................................
P. l. hacketti   ...G.........................C..........TC
P. l. pearsoni   ...G............T............C..........T.
P. penicillata   ........-.GC....T-.....T..T.C...........T.
P. herberti      .........-CT....-.....C..T.C...........T..
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Fig 1 A number of DNA sequences from seven species of rock wallaby. The sequence at the top is the Depuch Island rock wallaby used in this study, and this has the greatest similarity to *P. lateralis lateralis*. The similarity can be seen by the dots below the top sequence, which represent the same sequence. The main point to note is that there are lots of different DNA sequences in the other rock wallaby sequences.

RESEARCH

be replaced to reconstruct the skull, as the specimen did not have to be destroyed, so from a morphological perspective the process can be carried out without the need for sample destruction (see Fig 2). Furthermore, this type of approach to conservation biology will become more widespread, because the subfossil and museum material may represent a large proportion of, or in many cases the only, material that is available to work with in Western Australia. From another conservation viewpoint, this finding has also been very useful. The Department of Conservation and Land Management have removed foxes from the island (the main reason why the rock-wallabies disappeared in the first place), so it would be an ideal place to put rock-wallabies back. We now know which one to translocate.

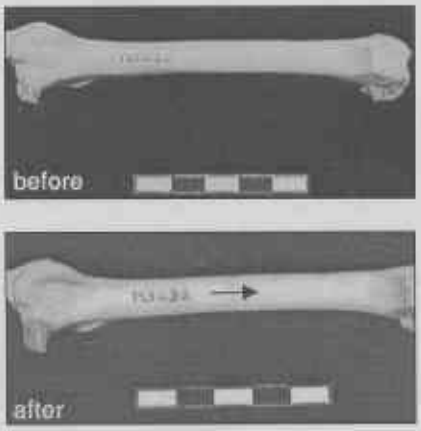

Fig 2 *A comparison of before and after the sampling of the femur bone from a museum specimen of the black-flanked rock wallaby from Depuch Island. It can be clearly seen that DNA analysis does not necessarily result in the destruction of museum specimens.*

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BUSH DETECTIVE

This is a real detective story!

Who made these little clay trumpets and what for?

Jarrad Hollins was walking through his bushland with Emma Bramwell (CALM Covenants) and Mike Griffiths (Woodland Watch) when they noticed these little trumpet-shaped structures. Jarrad thought they were built by termites - but *why*? Mike was so intrigued he ferreted out the story, see p 16.