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Legal Eagles — Wildlife Collections and the Law

Day 2

The Extraction of DNA from Old material

Alan Cooper, Oxford University Museum

What I would like to talk about this morning is everything we would do to a specimen after we have got permission from you lot to actually destroy it. So we will determine it is legal, you've got your Article 30 certificate and you have gone through the process of working out whether the destruction is going to be worthwhile, i.e. the information we are going to get from the specimen is going to be useful enough to justify it. I think Richard Thomas will be talking a little bit more later on how you might go about doing that. To give you a quick overview of the field of ancient DNA. This is the quagga, or was the quagga, an extinct zebra like species from South Africa and in 1985 this was the first extinct organism to have DNA recovered from it and analysed. From a fairly simple study, the quagga had only been extinct 60 or 70 years, the field jumped into the really big scheme of things, millions of years, with reports that DNA could be recovered from amber. The amber work has largely been discredited since that point.

These are the publications from 1984 on the left to 1998. We started of with the quagga, the Egyptian mummy, a human brain from Florida, everything was fine and dandy, we are looking only at specimens, we are back about a 1,000 to 3,000 years in age. Things went berserk about 1990. We had reports of around the 100 million year mark that DNA could survive that long, from Miocene leaves, termites, weevils, dinosaur bones, even bacteria. Basically I don't think any of those discoveries are believed in any more – I'll talk a little bit more about the problems a little later on. Then you have a range of other material, moas, that I have been working on in New Zealand, mammoths, cave bears, etc. The interesting thing is that all the reports which have been replicated or authenticated in some way go back in time to about this point. There is actually a theoretical limit based on the decay rate of DNA at about a 100,000 years and I suspect that that is how far we are able to go back with ancient DNA and optimal conditions. Optimal means deep frozen for a lot of these things i.e. permafrost.

What type of DNA are we looking for? You might think there is only one type of DNA and technically speaking you are probably right. We tend to talk about DNA from two different sources from within the cell. We have a nucleus in the middle which contains the genome. The little blue dots are mitochondria and these are what we call organelles, an analogy would be an organ in your own body. They are small semi-autonomous organs within the cell. They actually have their own genome and we tend to use those for ancient DNA studies because there are many mitochondria per cell, somewhere between a 1,000 and 5,000. Each mitochondria has, in itself, five to ten copies of it's own DNA. So, you are talking rather a large number of mitochondria copies of DNA in a cell. The nuclear DNA on the other hand – if you are after a gene which is present only as a single copy and most of the genes that really do anything particularly serious are present as single copies you are talking two copies in the entire cell. So if you had an ancient specimen and it only had one cell left in it and you are after a gene to study if you chose a nuclear single copy gene you have two chances in that whole cell of ever finding it whereas if you chose mitochondrial DNA you would have close to 50, 000 chances of finding it so you can see why mitochondrial DNA has been the gene of choice. There are multi-copy nuclear genes. They tend to be not too informative for phylogenetic studies or evolution. There is poten-

tial there for perhaps looking at individual identification but that is still largely unexplored.

So the mitochondrial DNA would be at least 90 – 95 per cent of the ancient DNA studies so far. The nuclear DNA has been used very rarely and I think no one is quite sure whether it has actually been successfully retrieved in anything older than about 60 to 80 years. The character we measure in mitochondrial DNA is generally the sequence of a small section or piece of the gene, whereas in the nuclear DNA, if you were doing something like micro-sattelite work you can either use the sequence or just the size of the product. Often that is variable enough between individuals to be used as a character worthy of study. Mitochondrial DNA is generally useful for studies in populations or particularly between species and above.

In the best case scenario when we are working with mitochondria DNA how much is actually present in an ancient specimen? If we look at some of the studies that have been done with Neanderthals or human remains where quantifying the amount of DNA is quite important in terms of proving what you are getting is the real thing. The estimate, I think, is about 200 copies for a 100mg bone and that seems to be both true for both Neanderthals and things more recent like the Anastasia remains. I suspect, however, that museum specimens may actually have a lot higher concentrations of DNA depending on how they are actually being prepared – I'll talk about the preservation in a minute. If you can imagine 200 to 300 copies of mitochondrial DNA and 100mg of bone and then the people who have been handling that bone, the curatorial staff, the people that are preparing it, the archaeologists the sweat on your hands or the breath that you are breathing all over the bone contain numerous fragments of cells that you have just shed. Each one of those cells has about 5,000 copies of your mitochondrial DNA so you can see that trying to work with some of the ancient specimens is going to be difficult. It is going to be a very small amount of the original DNA covered in masses of modern contamination and that is one of the harder problems with working with human remains. It is a little bit easier when doing studies on fauna, birds in particular, which is what I specialise in. But still it is important to realise how much DNA museums and archaeologists contribute to a specimen. Even in tissue or bone there are pores in the surface and basically you just get capillary action. The sweat is greased with bits of cell debris and that stuff is whipped up into these pores and your DNA shoots inside the specimen.

Where is the DNA preserved? There are a variety of sources of material. I prefer bone for several reasons. Firstly, you can clean the outside of a bone off with sandpaper and a drill and that will remove quite a bit of contamination. The DNA in a bone is largely protected from some of the processes that go on after death. If you consider DNA that is stuck in tissue, once the organism has died there are many enzymes floating around in your body mopping up anything that is foreign, viruses etc or enzymes which are contained within cells to do their jobs properly. After death a lot of that stuff is let lose and there is a massive period of what we call cortilitic damage when your own enzymes are chopping everything in sight. DNA in tissues is subject to that sort of action for quite some time, there is quite a lot of moisture in there and that is basically all the enzymes need and they are off chewing everything, DNA in bone, particularly in osteocytes (cells that are actually entombed in the bone)

[Slide]. – This is a cross section of a rhino femur from under Lloyds Bank in Trafalgar Square. It is about 30, 000 years old and has been preserved in the cold mud and clay of the Thames. Basically you can see after about 30,000 years enormous detail in the bone structure, the original (?) canals, interesting little flecks all over the cells. Particularly, within this structure you have osteocytes which are cells which are there to secrete calcium or reabsorb calcium in response to stress. So those cells are entombed in the bone and after death and not bathed in blood or liquid for the days or weeks it takes for the body to decompose so the DNA tends to survive a lot better. You may think that bone sample are going to be hard to come by, particularly from bird skins, for example, but quite often we find that the tops of the humerus are left in

prepared skins, you'll find fragments here and there or perhaps even toe bones (removed by a little incision from under the foot). If you can't get bone there are many tissues which will work reasonably well. It is best to sample from the extremities where this period of automatic digestion has been fairly limited just because it dries out faster. I find a particularly good source is from the thick skin on toe pads on birds but I suspect it will work equally well for mammals. No one has told me yet that it is a phylogenetic character so I have kept on using that and found it to be one of the better sources. The body skin on birds tends to be very thin and not contain a lot of cells and if you sample anywhere around the neck or the chest area both sites are going to be very close to the gut and there is a lot of bacteria in there which is basically going to go haywire after death so you find that a lot of that material has been quite damaged.

This is another specimen from New Zealand which is what I did part of my thesis on. It has fallen into a gap between two cave systems and basically got freeze-dried. It is between two large caves. They have a shared entrance and the caves have exits at different heights of the mountains creating a pressure difference which causes a draft to flow through the cave so there is normally a constant 30 mph wind going across this site at about 8 degrees C so effectively you've got a freeze-dried specimen. So what I did with this was to compare the various bits of tissue for DNA sources. You have bits of tendon hanging on the leg, dried muscle block on the pelvis, the bone. I figured we could actually have a look at some of this stuff and see what the DNA content was. Here are some of the pieces here, tendon fragment, skin, the rib which I have actually shattered with a hammer. What we found when we looked at it – this is a slide of an agrose gel which basically if you just think of it as a sieve that we are running DNA through. The larger the piece of DNA the slower it will progress through the sieve and we are starting at the top and we are coming to the bottom, we are using electricity to drive this. What I have done here is amplified a small piece of DNA, actually three different pieces of DNA. I've tried to amplify one a 147 base pairs, 200 and 400. The bone sample gave a very strong amplification when we tried to find a 150 base pair piece of DNA. The flesh didn't give us a very strong amplification. When we go to 265 base pairs the bone is still working well, the flesh – there doesn't seem to be much DNA of around 265 base pairs left in that specimen and by the time we go to 400 base pairs the bone is still looking very strong, there is nothing for the flesh. We did this in 1991. There had been a couple of reports that you could get DNA from a bone but they had all used human skeletons and when you get human DNA from a human skeleton, particularly when you use a technique that no one thinks should work everyone basically says it's your DNA. So this was authentic moa DNA which we had got from the bone and by that stage we started getting very excited about the whole potential of bone as a potential source of DNA. Excited because museums are full of bones! The tendon had a fairly low amount of DNA, the muscle was similar or slightly less than the skin. I suspect that you can correlate the amount of DNA in an ancient specimen with what was there originally i.e. tendon doesn't really have a lot of cells it's mainly elastic, strong helical structures, and the amount of liquid around the site and in the days after death how much damage would have been done by that. Feathers, the quill tip itself, analogous to a hair or a hair root, seems to contain all the DNA. Normally if I was studying a feather I would take off the first 2mm/3mm of the quill and that's all I would use for analysis, the rest of the feather doesn't really seem to contain very much at all. It's possible if you go right down the shaft you might find something but I think about 80 or 90 percent of the DNA is going to be in that first couple of millimetres and that's analogous to a hair root. You can get DNA from quite a long way from down a hair but again it's a very small amount, almost all of it is in the actual root.

Then there is the environment in which the specimen has been preserved. The best environment we find is cold, dry conditions e.g. alpine caves, technically permafrost is actually the best and there you can go back 30-40,000 years and get DNA out. That preserves DNA better than cold and wet which makes sense as the water is allowing degradation to occur, which is better than hot and dry, which is bet-

ter than the worst scenario of all which is hot and wet. So basically, bones from sand dunes on beaches are about the worst and bones from alpine caves are the best. The museum specimens which have been dried fairly quickly after death, haven't been treated with nasty things like varnish etc and then stored in a climate-controlled environment are very good. Not as good as the permafrost but getting there. The amount of time since death is relevant. I am not sure whether that is true due to enzymatic decay or just curatorial practice.

As a first approximation, the macroscopic structure the tissue or the bone is probably going to tell you how much DNA is going to be there as a rough guide. For a bone sample I am looking for a smooth, unglazed and uncracked surface preferably with some sort of greasy tone to it, a yellow, creamy sort of colour rather than a white, bleached sort of thing but you have really got to sample it to see if that is going to be backed up.

To sample a specimen and this is really of a lot of relevance to you. If you are working on human DNA this is the sort of stuff that we have to do. Your looking into a sealed laboratory that has got high-pressure air in there so that nothing from the environment can go in. You will be wearing a complete body suit, masks. The only bit of skin that is going to be exposed is your forehead and we are working on covering that up, we haven't come up with a solution for that yet. You've got little booties on. That's the sort of high tech approach when you are worried about contamination. What we would ask for museum curators doing sampling is, if possible, latex gloves and a breathing mask and that is going to stop two of the main ways in which you are going to contribute your DNA to the specimen. We would try and sample the interior of the specimen to avoid surface contamination. Particularly with bone I would abrade the outside with some sort of sanding disc. For bone, depending on the specimen, I maintain that about 3 – 5 mm cubed is sufficient for DNA analysis. It will depend on how your specimen has been preserved but I think as a general rule that's not too bad. When I'm sampling I try and avoid building up any excess heat because that will destroy DNA so if you are using a drill that will concentrate the heat at the point at which you are cutting so I tend to use a carborundum disc, a round disc about an inch in diameter spinning at high revolutions. That, because of the large surface area, will tend to cool as it goes around. It doesn't build up much heat at the cutting point. In studies that people were doing on teeth for example where they are drilling into a tooth there was so much heat building up that you couldn't touch the tooth, it was way too hot. The DNA recovery from that sort of technique is minimal and that's due to the actual drilling technique, not the amount of actual DNA that's present in the tooth. For tissue we routinely recommend about 2-3 mm cubed again is probably sufficient for most ancient DNA studies. In both of these cases we appreciate more if you can spare it but this is about the minimum we can get away with. You would remove that with a sterile scalpel, changing gloves between specimens and try to minimise the amount of dust or transfer from one specimen to another. You would avoid curatorial materials such as dyes and shellac.

What do you do once you have got the sample? The first thing is to work in a very isolated environment because, as I've outlined already, the amount of DNA in a specimen is minimal while the amount of DNA around is very large. That problem gets worse when your anywhere near a biology department because they are working with masses of DNA. Basically you can regard the floors of any modern molecular department as just a sea of amplified DNA products. Basically there are aerosol droplets going all over the place when people are doing pipetting or other actions within the lab, these things are floating around, dropping on the floor, drying out and becoming dust, everybody walks backwards and forwards picks these things up on their shoes and tracks them everywhere. So, to do ancient DNA work – to give you an example, when I was working in the Smithsonian, this is the National Zoological Park where I was working. Our lab was somewhere around here. That's were all the modern biology went on. To do the ancient DNA work I had to drive all the way up of the page here for about quarter of an hour to get to the lab where I do the ancient DNA work. That's the sort of separation

I regard as being practical. I would also, only do the work first thing in the morning, or in my case, first thing in the afternoon when you have just come in from home and you are wearing clean clothes, new shoes, you've had a shower. All the pieces of DNA that you have picked up from your modern DNA laboratory the day before have basically been shed at home, you've cleaned off and when you come in your not allowed to go to any area involving DNA work before you go to the Ancient DNA Lab and that applies for the whole day. Once you've been into any hot area your not allowed back to the ancient DNA lab. That's just trying to minimise the flow of contaminating DNA into your work area.

The laboratory requirements. Physical separation. Temporal is quite a good one if you can do the work on the ancient specimen before you do the work on the modern species your chance of contamination are reduced. You use dedicated tools. This whole laboratory would have completely separate equipment, protective clothing and routinely using bleach to try and get rid of anything that has managed get into the room. Also controls through every step of the process is very important.

How do we go about getting DNA out. You can use several techniques because certain specimens will present certain problems. Insects seem to have a large amount of strange polysaccharides in their skin and other areas which will get in the way of DNA but behave a little bit like DNA and tend to be isolated with it and then get in the way of all your enzymatic processes later on. You have to use a certain process, C tab(?) is the name of it, to get DNA out of insects. C tab (?) is used commonly in plant material as well. In general for vertebrate specimens I would mechanically chop the specimen. If it was tissue I would chop it up with a scalpel, try and use low heat. This is a parrot sternum from the four-corners area of New Mexico and this is a technique I use. I'd break it down to bone powder and place it in a chemical called EDTA – it's a chemical that likes calcium and will pull calcium out of that bone matrix, leave that overnight mixing well so by the next day a lot of calcium has been pulled out of the bone is starting to look quite gooey. At that stage I would treat the bone just like I would treat tissue – you digest the specimen, you stick in the proteinase which is just an enzyme (I think they're putting it in detergents for washing machines these days), a strong detergent and then you would gently mix it at a reasonably high temperature. What is going to happen is a proteinase is going to chop down those cellular components releasing the DNA and also fats and carbohydrates and anything else that was left behind in the cell. You then extract the DNA from that mix using, commonly, what we call an organic solvent method. Basically you use phenol and then chloroform and what happens is you mix the phenol with your mixture of x cell and centrifuge it and you have an aqueous and non-aqueous layer. The protein products shoot down into the non-aqueous layer, the DNA tends to hang around in the aqueous layer. So if you wash it twice with phenol and then once with chloroform your DNA will be in water reasonably dilute. There are another couple of techniques that you might hear. One is a silica method which basically exploit the fact that DNA will bind to silica in conditions of high salt concentration. That's a technique that is good if you are worried about other components present in your specimen which are going to inhibit your ability to grow DNA later on – things like arsenic, lead, various salts that have been rubbed into specimens. I find, however, it doesn't give me nearly as much DNA as my organic solvent mix so I tend not to use it so much. The last method is a very quick and dirty thing called KX (????), you might hear about. This is basically just a compound that collates, i.e. it binds cations, things like calcium, magnesium, positively charged ions. Wnzymes that digest DNA (called DNA-ases generally) very commonly require magnesium or calcium to be activated, so all your doing with keelex (??) you boil the specimen with these beads and they bind all the magnesium and calcium around preventing the enzymes from chopping anything up. Unfortunately you haven't removed the enzymes you haven't removed anything at all, you've just stopped them working temporarily, so you find many people that do keelex preps very quick, very easy just takes 15/20 minutes, find that in 6 months time that they have no DNA left at all. Basically what's happened is at some stage they've opened the

tube or somehow, a little bit of calcium or a little bit of magnesium has got back into that system and bumph! Everything's off again. And your DNA will get broken down over time. So this is really only if you're not particularly worried about your specimens a lot more than you just want something quick and dirty.

In all three methods you'd have your DNA isolated,. You can recover it with alcohol precipitation either for centrifugal dialysis, especially if it is through membrane and you centrifuge it, the DNA can't fit through the membrane but everything else can, it just sits on top, so you just spin everything else away and just concentrate your DNA. When you extract your DNA you must carry out control extractions. This is very important because of the risk of contamination from modern DNA everywhere. You must extract nothing i.e. stick two blank reactions in there and do all this stuff and try and isolate DNA even though you haven't put any actual material in at the start of the experiment. That's very important to find out how many of your components, your phenol, your chloroform or anything else might have DNA in them, which would give you anonymous readings. To do the extraction you should be using hi-tech safe equipment, I worked in New Zealand where we don't have COSHH standards and this is a converted chart recorded and all I doing there is spinning the bone powder in EDTA overnight just at slow speed. You can see the sort of colour of dirt and gunge you're getting out of the bone material.

So now we've got the trace amounts of DNA back from the ancient specimen, how do we turn that into something that we can use? The whole field of ancient DNA is basically revolutionised by the concept of PCR. PCR is Polymerase Chain Reaction. Polymerase is basically just an enzyme that copies DNA and Chain Reaction is referring to the iterative process that is used. It's fairly clever but relatively simple. There's two pieces of DNA here or if you consider a gene that you're interested in and there is the DNA the two strands are bound together in the normal DNA structure. Now you're interested in this little highlighted area here that you know already exists because someone has already found it in a frog, for example. What you do is design a little synthetic piece of DNA, which you'll get made in a chemical company called a 'primer', a PCR primer, and that's this little short piece here. What you've done when you've designed that is made sure that it will match a little piece here and here of your existing gene. What it is going to do is it is going to stick to it, it is going to recognise what it has been designed for and it will locate it amongst the entire mish mash of DNA and find just that spot and join onto it. So what we do is take the original piece of DNA, you heat it up to about 90 degrees at which stage it will separate. The two strands can't hold themselves together at that temperature and now you've got this piece here and this piece here. You then cool it down, as you cool it down the DNA will want to join together. If you put enough primer, in the primer is going to get to the strands of DNA before those two find one another, so the primer is going to join on here and join on here. What happens then is the polymerase, it's job in life is basically to recognise a double stranded piece of DNA, that becomes single stranded DNA and fill that hole. It's there to repair damage from UV light or errors in your own copying when your cells are dividing. It's there to fill in holes and prevent errors. So it sees this nice double stranded piece here and it sees the single stranded stretch and it thinks, right better fill that in, that's going to be dangerous, so it copies this strand all the way down, filling in the appropriate piece of DNA that is complimentary to it, i.e. A, T and G will bind with C, copies it all the way down there and copies it all the way down here. You've now got one, two, three, four pieces of DNA, whereas before you only had one, two and they will be the same sequence, this sequence here will be the same as the original one there, and that is going to be the same as this. You then heat it up to 90 degrees again, and separate it and now each of those four strands becomes a template, one, two, three, four, you cool it down the primers join back on and you go through the process again, they copy each of those four strands and you now have eight. You do that about 40 times and you end up with many hundreds of millions of copies of that one short piece of that DNA you've been inter-

ested in. That is important in all sorts of stuff, forensics, medicine anything involving DNA, but it really enabled ancient DNA to become a technique that was relatively easy, because you could take the one or two copies of DNA that were left in a very old specimen and turn them into many hundred million copies which you could then use to study.

To give you an idea of how you build a large piece of DNA, this is the moa mitochondrian genome that I've been working on at Oxford. The genome means in the mitochondrion the entire section of DNA that the mitochondrion uses is about 16,000 base pairs this is 16 and that's zero. What I've done is design lots of little primers, these little things here to amplify small, short regions of that lower DNA and by doing this on and that one and that one and this one, I'm actually building up the entire sequence, I've just overlapped them and this is the sequence that has been generated so far, here to here, got a gap there, something didn't work, and then here to here and then here to here. So while ancient DNA has traditionally used only very short pieces of genes, maybe 200, 300 base pairs, people consider that's so difficult, that's going to be enough, unfortunately it may not be enough data. It might be enough work but it's not going to be enough data to make any real conclusions. So what I'm saying here is we'll just ignore all the dogma, and what we're going to do is to sequence the entire thing. I've designed primers right the way across, there is a gap here, I haven't gone between 9 and 14 yet so I've still got about half of it to do, but this has taken about three months, to do those three sections, so I should be able to knock it off before August. That would be the first mitochondrian genome of an extinct species. Technically, if you're really interested, you could then synthesise this protein, knowing the sequence and find out if it worked. That would tell you if we are accurately recording information from the past because if it didn't work you'd know there was a problem, the bird certainly lived at some stage, the proteins had to work. You could synthesise the whole thing too but it would cost a lot of money and I couldn't see the point of it. Those are fun things you could do if you had too much cash. I'm referring obliquely to a couple of Japanese scientists that are thinking of doing this sort of stuff.

So, PCR is very powerful. You take one copy of DNA and you turn it into a hundred, million copies or so. But it's very, very sensitive and that's because of contamination. While I've got one copy of moa DNA, I might have had a hundred copies of human DNA sitting around it. I got to make sure I get the right thing and not human sequences right throughout. So that's one big concern. People take that far too lightly when they do ancient DNA studies in general. You find people working in the same lab as modern PCR experiments. They'll be sitting in one corner trying to study one copy of DNA, a guy three feet away has generated a hundred million and is just spilling them over the bench and people think this is acceptable. You must do a control PCR reaction, probably more important than the extraction control because here you know that anything is going to be amplified and this is how you get results from amber insects and everything else. Basically it is very powerful and therefore you have to do preferably two controls for each time you try and amplify something to make sure there is no DNA in the system. To authenticate it when you do get a DNA sequence e.g. out of a dinosaur bone, the first thing you've got to do is check the sequence of DNA makes sense. This is the first thing the people in America didn't do, well I suspect they did and it didn't make sense, and didn't report it in their paper, because when you analyse a sequence it came out next to humans and cows I think, so fairly obviously that was mammalian contamination. It should be ideally a novel sequence particularly if your specimen is extinct, i.e. there shouldn't be any DNA from a quagga floating around in the world today, so the sequence you get from a quagga should look different from anything before, although preferably closely related to a zebra, and you should have blank controls, that's very important. These were the criteria that we used to use until about the early '90's when some of the amber stuff started coming up. At that stage people started demanding replication. If I say I've got dinosaur DNA, I'm not allowed to publish that until I get another laboratory to independently sample that specimen and also get dinosaur DNA and have the same sequence that I've

got. Once that criteria came in in about 1993 there have been no more reports of million year old DNA.

Then you've got things like histology. This is where you've still got a DNA sequence. It seems to make sense, you can't disprove it that way. It's a good idea to go and look at the specimen. Work out, for example, if it's bone? Is there any macrostructure? Can you see collagen fibres in there. Does it have good histology? Can you see the cells that I showed you before? There is a scale of one to five which some people on Oxford Archaeology Group have come up with to grade how well bone is preserved. Anything greater than two is generally accepted as being possible for DNA analysis. You can see how much nitrogen is left. The percentage of nitrogen in living organisms is about four to five percent. Anything over three percent in an ancient organism is encouraging. Then you can study things like rasamisation which is how proteins have decayed through time. If there are minimal amounts of decay you might be excited. You can do things like carbon dating. Basically, if the specimen is older than 11,000 years and it hasn't been preserved in ice or very cold conditions I think you're going to be very suspicious if you got DNA out of it.

Now you have got your sequence, you've authenticated it as much as possible. What can you actually do with it? Originally it was all phylogenetic studies or anthropology, e.g. the quagga – how would that fit within zebras? How are these ancient humans related to modern humans? The million year plus club turned up and they all tried to study evolution over a very long time span. You've got the saber tooth cat, how that fitted in with the cat family? Moas, cave bears, ground sloths, mastodons. Those projects are much easier are going to be a lot easier because your risk of contamination from modern humans is going to be minimised. Things like Neanderthals, the iceman are much more difficult but have still been done.

I want to talk about the study on the Laysan duck which is one that I have been involved in because that had some legal implications which were slightly interesting. The Laysan duck is a threatened species that has varied between 20 individuals and about 500 in the last 60 years. It wanders around, amongst the sand dunes on a remote atoll of the Hawaiian island chain called Laysan Island. Very small, it's about 270 hectares. This atoll is basically a large lagoon surrounded by a thin strip of coast with very little height. It's very isolated, small population and one disease or hurricane is probably going to remove the species completely from the wild. You think that there would be a desire to set up another population so that you have an insurance policy for that species but the politics of introducing things to Hawaii are very sensitive because so many of them have been done badly and have gone completely wrong like the mongoose. So, at the moment, the Fish and Wildlife Department couldn't do this, there would have been a legal challenge and therefore, the conservation management of the Laysan Duck has remained in limbo.

This is what the Laysan Duck largely does. It feeds on the flies that live on the lagoon and people thought that this was a fairly specialised ecology, you don't see that too often. A lot of the conservation plans have been based on this behaviour of these ducks. On the main islands of Hawaii I've been working with Helen James and Sors (?) Olsen doing a lot of caving there, finding old bird bones. What I did notice was that there was a duck, particularly from high altitude lava flows where you have caves. That duck didn't seem to fit anything else that was known from Hawaii today. It was a little bit larger than the modern ducks – the mallards and co. Ecologically it didn't make sense. What sort of duck lives in very dry lava fields? They couldn't identify it so they suggested that perhaps it is something related to the Laysan duck and that we might want to have a look at it from a DNA perspective. We extracted DNA from bones that were 1,000 – 2,000 years old. Quite poor condition in general, they were fairly fragmentary. We extracted DNA after a bit of effort and found, interestingly enough the DNA from bones fit right in with the modern Laysan duck population where there is no mitochondrial DNA variation whatsoever. These are the places in the sequence where you get variation between the Laysan duck and other possible out-groups

and these guys match up. A phylogenetic tree based on that shows you the bones grouped very tightly with the Laysan duck and actually revealed that there was a little bit more variation on the paths, just different form of DNA which is now not seen in the modern population – they're down to one form. It is quite separate to all the other ducks on Hawaii. Because we can show that the Laysan duck was formerly distributed all over Hawaii at high altitude the Fish and Wildlife Department now has some sort of defence to say that we can actually reintroduce this bird to Hawaii. It was there formally. The Hawaiian natives had exterminated it when they turned up but formally it was part of the ecology. You don't precisely know what it is going to do because the ecology has changed since it disappeared but you certainly know it had a role in the original one and one of the islands they are reclaiming from the military might be used as a source for setting the Laysan duck back up in Hawaii. I think most importantly, it showed that peoples views of how you should conserve an isolated island endemic by feeding it brine flies and keeping it on a little atoll had nothing really to do with what that duck was originally adapted for. It was a high altitude specialist ranging right up and down the Hawaiian island chain. It probably had quite a diverse set of ecological niches it could have exploited so basically when you come across small populations in the Pacific quite often they are remnants of a much larger widespread group and really you should take that into account when trying to plan what ecological climate they are going to have.

One last thing. I would just like to say from the ancient DNA perspective we are all very reliant on the museum curatorial staff to give us our samples and basically we can't do much more than acknowledge the museum in all the publications we get but without you guys we can't do our work. What I have done in a couple of cases is about the only thing I can contribute back is write letters of support and things like this during rounds of funding cuts and we've done this a couple of times in various museums but basically we will do what we can but in the mean time we are totally reliant on you guys.

DNA from Museum Specimens

Mark Wilcox
Liverpool John Moores University.

The work I am going to present is work that has been done with Dr Malcolm Hall from Liverpool University, Dr David Mellor from Liverpool John Moores University, and I am grateful for the assistance of Dr Clem Fisher from Liverpool Museum and Dr Andrew Kitchener from the Museum here in Edinburgh.

We first became interested in what we could get from museums when we started to look at some bones that were collected from Furness Head. This [slide?] is a piece from a rib bone from an unknown species, although it was almost certainly a feline. These bones had been excavated from a small crevice that had possibly, in the past, been part of a cave system. The bone had been completely mineralised on the outside (ranging from about 0.1mm to 0.01mm). On closer examination of this bone we found a number of quite interesting objects, notably this cell here[slide]. This doughnut shape and characteristic size of about 10 microns made us think that these looked very similar to red blood cells.

We did some electron dispersive micro-X-ray analysis of the bone and found that the interior of the bone gave us readings which were very similar to contemporary bone in terms of the elemental composition, including calcium, magnesium and iron. When we scanned the cells themselves, we found a very high level of iron compared to the background and, again, this made us think that perhaps what we had were blood cells. If you look closely you can see that there is some damage to these cells. We were rather curious about this damage until we started to look at fresh blood cells, which we also scanned using EDXA. We found that the X-rays actually made a very similar damage pattern to those fresh blood cells, so we had another look at the bone. This time we didn't use EDXA, and found that some of these cells were completely