The Biology Curator

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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

undamaged. This led us to the perhaps startling conclusion that from this bone, somewhere between 5 – 7,000 years old, we've got tissue in a very good state of preservation! In fact, we may even have tissue in a soft state, despite the hard, mineralised exterior. We became quite interested in this and decided to try and extract some DNA. This is a multiplex PCR to amplify DNA, using specific primers for feline cytochrome B. This [slide] is a fairly large fragment, about 400 base pairs, and a much shorter fragment of about 80 base pairs at the top. It appeared to us that we could actually get DNA from bones that were about 5,000 years old. This led us to start thinking that we could use museum collections.

I'd like to just summarise the procedures that are involved. One has a DNA extraction method which is dependent on the sort of source material that you are using, be it feather, tissue or bone. After you have got your extract, you then go on to the PCR step and then you do your double stranded sequencing, and finally down to your analysis. The two stages that I would like to concentrate on are the DNA extraction and the PCR stages. From the museum perspective these are the critical stages.

We were wanting to use DNA from museums for a couple of projects – Amazon parrots and Psyllids, a small insect which people are interested in terms of potential global warming and speciation questions. When we started to use these specimens, we found our task far from straightforward. Our hit rate, the chance of getting amplified product, was not 100 percent. After consultation with colleagues working in other labs, we found that this wasn't actually a problem specific to us and that a lot of people had problems getting DNA. We decided to take a very basic look at what was going on. We figured that there were two problems. The first one was the preservation of DNA and the second one was possible inhibition of DNA extraction or PCR steps by chemicals used in preservation of the sample.

There is probably little we can do about the preservation with the current technology. If the DNA hasn't been preserved particularly, then that's it, you've got to use another method to answer your question. There is some work going on with the use of lygases which actually repair DNA, although the likely results of those studies are going to be controversial if they are used for phylogenetics. There is also work using tunnelling electron microscopy where you actually look at the DNA directly, although the state that is at is far from being ready for use in widescale genetic projects.

There are other possible solutions to preservation problems. You can use mitochondrial DNA. The copy number of mitochondrial DNA far exceeds that of nuclear DNA and also, because it is a closed circular molecule, it's preservation is much better than nuclear DNA. Another method is to use overlapping contigs. These are essentially very small products that you amplify, typically of the order of a 100 or 200 base pairs. From fresh material, it is not unusual to be able to get 10,000 base pairs very easily and when we are talking about museum specimens we typically find that 100-200 base pairs is a fairly modest target.

Other alternatives include taking multiple samples from the same specimen or, if the specimen is well represented in a collection, from different specimens. This sometimes brings us into conflict with curators because there is obviously a great deal of emphasis on keeping samples for future studies and this sampling tends to be destructive. Taking multiple samples is actually quite important. This slide here shows some sequence from a Thayer's gull feather. The feather is about 60-70 years old and it is a single feather. What we find from this single feather, on a number of amplifications from different samples, we have got a conflict here. The sequence is very, very different at one point to that at another and you can see that in the alignment of the bases.

When DNA is damaged, and the damage is widespread, then at the PCR step you tend to get a complete failure. So those damaged DNA fragments fail to replicate. However, if the damage is actually quite slight, perhaps

just one or two missing bases, polymerase can still travel along that strand copying it and so you don't get a proper replacement of your damaged template from your final pool of PCR products. At the end of your PCR step, when you come to sequence and actually read that DNA, you can find that you have got these spurious sequences in your sample. You also have to be very careful with the polymerase, because there are different fidelities associated with different brands, as it were, of polymerase. The fidelity is the accuracy with which that polymerase copies the original target DNA.

Going on to the second problem with museum specimens. This is one of inhibition, and different preparation methods can result in inhibition of enzymes used in either the first step, the DNA extraction step, or in the second step, that of PCR amplification. And, of course, some preservation methods are actually detrimental to the survival of DNA for future studies. It looked like this was going to be the step that we could actually make some headway.

We got a supply of different samples of bird and mammal skins which had been treated in different ways. Using a control, which was simply a freeze-dried mouse skin, and using this EDXA technique, we started to have a look at what elements were present. We expected oxygen, phosphorous, sulphur, chlorine and potassium, because these are just normal elements that you would expect in skin samples. Anything that differs from that control is likely to have been introduced during the preservation technique. We found it quite difficult to relate preservation techniques of, say, arsenic treated skins with the final elements that we detected. Because so many museum skins don't have very good records about preservation, especially those preserved in the last century, this was a problem.

What we did was to take samples of those skins, duplicate them and cut them in half. Half went for EDXA treatment, looking at the elements. The other half we soaked in water for 48 hours to produce a rinse water, to look at the water-soluble compounds that were in those treated skins. We then took the water treated samples, removed the water and introduced a known amount of protein ova albumen together with protinase K, which is a typical enzyme used in DNA extraction, and looked at the action of protein hydrolysis over time.

Obviously, at the start of the experiment we had a 100 percent of protein and, as the experiment went on, some of that protein was digested [slide]. (These samples here relate to the samples on the previous slide with the different treatments). Using an unbuffered DNA extraction protocol which is not dissimilar to many which have been used in published papers, we found that there was very little digestion in some of these samples and more in others. This is the untreated sample [slide], so that you can see that in unbuffered conditions we've got about 20 percent digestion. There was a significant difference between two groups of skins. It looks like some of those treatments do prevent maximum utility of protinase K in the DNA extraction stage. We then used a buffered experiment using EDXA and kelax(?) and we found a tremendous improvement in the ability of protinase K to digest protein. As you can see [slide] we have got a great deal more digestion going on here. What we had was a picture where some of these preservative methods do actually hinder the DNA extraction stage, decreasing the effectiveness of some of the enzymes we might use to break down the sample to release that DNA.

The next stage of the experiment was to take some of the rinse water and introduce it into a PCR reaction. We took a known amount of DNA from a plasmid and tried to amplify a very small gene, the laxed(?) gene – about 370 base pairs, by using the rinse water on two different concentrations of target DNA, of the order of 10^5 and 10^8. That is to say, the first one has 10^5 copies of DNA and the second one has 10^8 copies of target DNA. We then performed the PCR experiment and found that there is a big gap where we are getting no product. We know that we have got good DNA and we know that our primers are a perfect match, but what we are finding is that there is some inhibition on the treated museum specimens that is preventing that DNA

polymerase working.

We are now trying to go on from this work, using an ion exchange resin to try and clean up the DNA from museum specimens prior to both the DNA extraction and the PCR stage. We are also using EDXA and mass spectrometry to try and fingerprint museum samples and this will give us an idea of the chemicals used in preservation. Then we will be able to relate that to possible inhibition effects and be able to try and solve that by different clean up methods.

We are also working on repeated, non-destructive sampling for DNA. One of the projects we are looking at involves bird skins. Many of these skins do not have bones and we have been using feather, with about a 30% hit rate in terms of amplifying the DNA. What we are now trying to do, rather than taking the feather off the skin, is to actually do the DNA extraction on the skin by introducing the extraction buffer through the shaft of the feather and incubating the skin at a slightly elevated temperature of about 35 degrees. By doing this, we can sample several feathers and only put a very small whole in the feather shaft. The results from this seem to be quite encouraging, but that is as far as we have got at the present time.

Guidelines for Destructive Use of Biological Material

Richard Thomas
Natural History Museum

I'm going to talk about guidelines for the destructive use of biological material. Effectively there are two versions of this talk I could give you. There's the short version. There is really no difference in principal between destructive sampling of specimens for molecular work and any other kind of destructive sampling. In fact, as you have probably gathered from some of the stuff that Alan was saying earlier, destructive sampling for molecular work is sometimes much less destructive than some of the techniques standardly used by morphologists when they are doing some of their techniques. I'll give you the slightly longer version of the talk which is derived from an article in a now extinct publication called 'The Ancient DNA Newsletter' six years ago, and written by ????? Havow (?), Bob Wayne and myself and much of the material in that article has subsequently been incorporated in the NHM's policy document on sampling for molecular purposes from the collections.

The somewhat longer version. I think we need the somewhat longer version because there is this cultural difference between molecular biologists and museum curators. Curators often see molecular biologists as sort of evil interlopers who soak up valuable resources and take up space that could be better used for storing collections. Some of the molecular biologists see curators as traditionalists who don't recognise or are incapable of recognising the path-breaking importance of their research. There needs to be some way of mediating between those two sorts of cartoon extremes. Specifically you need criteria for evaluating requests for the use of material and that pre-supposes having somebody around who is qualified to evaluate the requests. You also need to consider what a museum or holders of a collection should expect to get back from a loan of material.

In 1992 we came up with five criterior for evaluating requests for destructive sampling of specimens. The scientific value and the feasibility of the project, the qualifications of the investigator or the lab to do the work, could they possibly get this material some other way other than destroying specimens like from captive populations or wild populations. The volume of the material required relevant to what is in the collections, so if they are going to grind up half of the single existing individual of something it would probably not be a good thing. And finally, the staff effort required to fulfil the terms of the loan. I will go through all of these in slightly more detail.

Feasibility and scientific value. Is it of sufficient interest to justify the damage done