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Episode 61: Deconstructing / Reconstructing the Tasmanian Tiger

Deconstructing/Reconstructing the Tasmanian Tiger

VOICEOVER
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SHANE HUNTINGTON
Hello and welcome to Up Close coming to you from Melbourne University, Australia. I?m Doctor Shane Huntington. On September 7th 1936, in the Hobart Zoo based in Tasmania, Australia the last known living example of the Tasmanian Tiger - the Thylacine - died.
The Tassie Tiger is one example of the extraordinary flora and fauna found in Australia, but unfortunately is also an example of the myopic thinking of the time. On November 4th of 2008, Michael Crichton the author of Jurassic Park died. Although not hunted to extinction, Michael Crichton is linked to today?s discussion by his fictional writings about the resurrection of long dead species using genetic tools.
The Tasmanian Tiger is not a dinosaur, it?s a marsupial, but our fascination with this creature has led us to the resurrection of at least part of its biology.
Today on Up Close we are joined by two of Australia?s leaders in marsupial research; Professor Marilyn Renfree, from the Department of Zoology at the University of Melbourne, Australia and Associate Professor Andrew Pask, Molecular and Cell Biology, University of Connecticut, United States, who joins us on the phone. Welcome Marilyn and Andrew.

MARILYN RENFREE
Thank you.

ANDREW PASK
Thank you.

SHANE HUNTINGTON
The Tasmanian Tiger, or as I?m sure you call it the Thylacine, was a very unusual

animal, and as we know, it was hunted to extinction in the earlier 1900s. The last known living example, as I mentioned, died in the Hobart Zoo in 1936. Andrew, can you tell us what this creature was like? How did it fit into the food chain?

ANDREW PASK
The Tasmanian Tiger is a really interesting animal because of all of the marsupials it?s the one that most looks like the other mammals that we?re more familiar with, in that it was so closely resembling that of a dog. So the skeleton formation of the Thylacine is actually one of the most striking examples we can see within different evolutionary states of an animal that looks very similar to a dog.
It?s interesting and why it evolved that particular look and that particular skeleton is because it has a very similar diet to what a dog would have. So it?s a carnivore. It would be required to cross large distances of ground.

SHANE HUNTINGTON
Andrew I understand this is a good example of where we see this phenomenon ? convergent evolution?

ANDREW PASK
Yeah, that?s exactly right. Convergent evolution was a term that was used when different animals from different lineages, or different groups, have actually developed along the same pathway. So they look the same, even though they are quite different animals, so things like a bird wing versus a bat wing. So birds have wings and also bats have wings and they?ve just come to that same evolutionary outcome. It is similar here, that we see a marsupial developing a body type that is very similar to a dog.

SHANE HUNTINGTON
Andrew the particular memory we have of the Tasmanian Tiger is that it was last located in the state of Tasmania, which for our international listeners with a map in front of them, is the very southern point of Australia and it?s detached. It?s an island, a little bit of the coast.
But it was on the mainland for a long time and in New Guinea, I understand. The conditions must have changed for it, in such a way, that it became extinct on the mainland and was forced into Tasmania. Can you say a bit about that?

ANDREW PASK
There are a lot of different theories as to why they think it got wiped out on the mainland of Australia and also in Papua New Guinea, but one of the main theories is that probably the dingoes came down into that part. So they introduced domesticated dogs and that simply occupied the same niche as the Tasmanian Tiger and eventually led to that being removed from that particular spot within the food chain.

SHANE HUNTINGTON
Marilyn, were there any other environmental conditions that drew it to that point?

MARILYN RENFREE
It?s hard to say. There is a large deposit of Tassie Tigers in a cave in Western Australia called Devil?s Lair and some of you might know the Devil?s Lair wine label that has been recently picked up by some of the Margaret River vineyards.
But Devil?s Lair contains a great many Tasmanian Tigers, so it?s clear that they were spread right across the continent and they became extinct on the mainland?well actually it?s hard to say exactly when they became extinct because we have such a relatively poor fossil record in Australia. The best explanation we have is with the arrival of the Aboriginal peoples and the introduction of the dingo as a dog competitor.
But Tasmania and Kangaroo Island and some of the other offshore islands separated from the mainland of Australia about 10,000 years ago. That was very fortunate because, in many ways, there are lots of species in Tasmania that we still have that are extinct on the mainland largely due to our efforts because we introduced the fox and the rabbit and many, many, many other feral species.

SHANE HUNTINGTON
Despite its appearance, it?s neither a dog nor a tiger; the Thylacine is actually a marsupial. Marilyn, give us an idea why marsupials are so unique.

MARILYN RENFREE
Marsupials represent, I suppose, about 6% of living mammals. There is about 5,000 species of mammals. Marsupials were very diverse in the Cretaceous period of geological era and they were very abundant in South America.
So there were both large and small mammal niches occupied by marsupials in South America. There was, for example, a sabre toothed marsupial tiger called Thylacosmilus. In Australia there was a marsupial lion called Thylacoleo. These were very large, large animals.
With the separation of Gondwana; South America, Antarctica and Australia separated as the continents drifted apart and India scooted up to bang into the Himalayas. The marsupials that had made their way across into the Australian mainland thrived and expanded. Whereas in South America the mammals from North America were able to migrate south and for reasons that we still don?t completely understand, most of the large marsupials became extinct in South America, but there are still many, many species ? about 66 species ? of marsupials that are found in South America and they are all small marsupials.
But we know marsupials best from our iconic kangaroo, of course, on our coat of arms. The kangaroo has a pouch ? not all marsupials have a pouch ? most Australian marsupials have a pouch. Many South American marsupials do not. The pouch is really like an exteriorised uterus, so marsupials give birth to young that are relatively undeveloped at the time of delivery. So they?re more like an exteriorised foetus and they complete their development within the safety of the pouch, attached to one of the mammary glands, to a teat.
So that a kangaroo or a wallaby, that we do most of our work on, has one month pregnancy and nine months lactation. It?s pretty much the same period of protection that we give our intrauterine embryos, but instead of it being in the uterus it?s in the pouch, and I always say that since marsupials give birth to such tiny young I know

which I?d rather have given birth to.

SHANE HUNTINGTON
Indeed, my wife had a 9 pound 2 ounces.

MARILYN RENFREE
Well my second was 9 pound 10 ounces.

SHANE HUNTINGTON
There you go, we have a winner. Now I understand in the Thylacine we have the unusual situation that the males also have a pouch.
MARILYN RENFREE
I don?t think they do.

SHANE HUNTINGTON
Okay.

MARILYN RENFREE
The cremaster muscle, that all mammals have, is a muscle that goes from the pelvic girdle down into the scrotum and when it?s cold, if you look at a kangaroo on a cold day - I won?t draw any analogies with human?s ? but if you look at a kangaroo on a cold day you won?t see his scrotum at all, it?s pulled right up into the body. If you look at a kangaroo on a hot day you will see, indeed, that he is a male kangaroo.
So the arrangement of the cremaster in the Tasmanian Tiger is up in the same region as where the pouch is because marsupials are also different from other mammals, in that their scrotum is on the head side of the body not the tail side of the body. So it?s exactly the opposite arrangement of penis and scrotum to all eutherian mammals.
So the scrotum is much in the same area as the pouch, so it?s on the abdomen. In fixed specimens, or dead specimens, the muscles are going to contract and there?s really just like a little bare part of the fur around where the scrotum is.
So my opinion is that it is not a pouch, it?s really just where the scrotum is going in attaching to the body.

SHANE HUNTINGTON
I?m very happy to have that urban myth corrected for me. Andrew, can I ask you what the current closest living relative is to the Thylacine?

ANDREW PASK
The only one we have around at the moment is probably the Tasmanian Devil. So when we?re looking at the genetics of the animal, which is what we try to do and try to understand it in a lot more depth, the closest living relative that we can compare it to would be the Tasmanian Devil.

SHANE HUNTINGTON
Tell us a little bit about the Tassie Devil. Describe its appearance, because I think people would be quite surprised how different it looks to the Tasmanian Tiger.

ANDREW PASK
Well to start off with the Tasmanian Devil is a lot smaller than a Tasmanian Tiger. So it would be equivalent, I guess, in size to a small dog and it?s also got quite a different morphology. It?s not your typical dog shaped animal; it has got a very large head in the comparison to its body size, very large jaws, quite a small carnivorous marsupial. So it?s in the same group as the Tasmanian Tiger, but they are quite diverted, they are not extremely closely related.

SHANE HUNTINGTON
Andrew how far back in history do we have to go ? we?re all mammals ? before we find some common ancestry between us and the Thylacine?

ANDREW PASK
Between marsupials and eutherian mammals ? which is the group that we belong to - you?re looking at somewhere between 120 to 140 million years ago. So that?s the time when we believe from a combination of genetic resources and fossil data that we think the two lineages diverged. That?s a very long time ago, which makes them a really interesting group genetically to compare the genomes between us eutherian species and marsupial species and really start to find out which genes we have in common and what really it is that defines the mammal.

SHANE HUNTINGTON
You?re listening to Melbourne University Up Close. I?m Doctor Shane Huntington and we?re speaking with Professor Marilyn Renfree and Associate Professor Andrew Pask about Australian marsupials.
Now recently you have managed to extract genes from a museum specimen of the Thylacine, insert these genes into a mouse and observe actual biological functions. This is quite a mouthful and I want to go through this step by step because I know there is a lot of science involved in each part of this process. Marilyn let me ask you first, how do you extract genes from a museum specimen?

MARILYN RENFREE
I?ll go back even further than that and then handover to Andrew because what we had to start with was obtaining the specimens. We are very fortunate that 100 years ago the traditional fixative for most museum specimens is ethanol and fortunately ethanol preserves DNA, whereas if it had been fixed in some of the other fixatives, like formalin, it would make it almost impossible to extract the DNA.
I was visiting a colleague at the Museum of Victoria because I was interested in looking at the marsupial mole and I was going through some specimens looking at the marsupial mole. In fact trying to ascertain whether they had a scrotum or not. The curator at the time, Dr Joan Dickson, said, ?You know I?ve got some Tasmanian Tiger pouch here.?
And I said, ?Fantastic. Do you think I might be able to get permission to take a sample from them??
She said, ?Yes, I think we can do that.?
So I did some paperwork and put in an application and was able to take some snips of tissue from four of the Tasmanian Tiger pouch young they had from one litter that

was held in the museum and had been preserved in alcohol, in ethanol.
We also obtained a couple of pieces of dried skin from adults at the same time, from the same museum. Both of those provided the starting source for the material that the DNA was extracted. Since Andrew did the DNA extraction, I?ll hand over to him.

SHANE HUNTINGTON
Andrew.

ANDREW PASK
So the DNA itself is well preserved in ethanol. But unfortunately as time progresses on during the life of the specimen the DNA starts to break up into lots of small little pieces. So what you?re left with, by the time we got our hands on the specimen after it had been in ethanol for about 100 years, was that the DNA in its genome now is cut up into a lot of tiny little pieces. So we had a very small array of very tiny little pieces of DNA.
So we were particularly interested in things that were important for the formation of a skeleton and really trying to find this gene that we thought we could get a biological function for and so we started to amplify small regions of that from the DNA that we were able to obtain.

SHANE HUNTINGTON
Andrew, given you didn?t have much information on the original storage of the particular specimen, how do you know for sure that the genes you extracted are actually from the Thylacine itself?

ANDREW PASK
One of the big problems with ancient DNA actually is exactly that. It is if you can really tell that what you?ve extracted definitely is ancient DNA itself and not just some sort of contamination that you get in during the process.
So what we were fortunate with, with the Tasmanian Tiger, is that although the DNA is very fragmented, so it?s in lots of small pieces, there is still a lot of it in the specimen. So we were able to get a very large amount of DNA out. Then once you actually get the sequences out ? so you?ve got your bit of gene ? you can actually then compare it to other genes from different species. So we compared the piece of DNA that we got to human DNA to make sure it wasn?t a part of my genome or Marilyn?s genome, and to mouse DNA, and to other marsupial DNA.
By doing that you can see how closely related that piece of DNA is to other species. We could see very clearly that that piece of DNA was most closely related to marsupials and was therefore of marsupial origin.

SHANE HUNTINGTON
Now I understand the next step is to take the DNA and introduce it into an environment that I suspect you would say is living, so a scenario where you can get it to activate.

ANDREW PASK
So lots of people have done a lot of sequencing of extinct genomes now. So there is

quite a lot of work that?s been done on mammoths and Neanderthal and what we could do up until now is really look at the sequence and try to figure out how similar they were to different species, or living species, that we know today.
From that you can infer a lot about an animal?s evolution and how it may have lived, but it?s very difficult to get a really good idea about how the genes work in development, unless you can actually look at them in a live situation. So how does this gene actually function and behave in a live cell?
So what our experiment was geared at was actually taking that piece of DNA from a Tasmanian Tiger, but then putting it into a living cell and into a living organism to see how that piece of DNA behaves. So we could actually figure out what the exact biological function was of that piece of DNA.

SHANE HUNTINGTON
So this enables us to actually look at a biological function in the exact same way it would occur in the original animal?

ANDREW PASK
It?s slightly different. So because all genes require other genes around them to help them work and activate and do their job, it requires that the mouse must also have the other bits and pieces that are needed for that particular piece of DNA to work. Fortunately, although we last shared a common ancestor with the mouse about?or the Thylacine did at least, about 130 to 140 million years ago, there still is enough conservation between our proteins and the Thylacine?s proteins, that there is cross-talk, so we can actually get the systems to work.
So it gives us a much better idea of exactly how that gene functioned and what that gene did. Obviously we?re looking at the function of that gene in a mouse, so it?s different to looking at it in a Thylacine. But it will tell us when that gene switched on during development and then which cell types switched on during development and that gives us a lot of information about then what we think it is that that gene does.
SHANE HUNTINGTON
Marilyn, tell us a bit more about the drive to investigate this. What sort of things can we learn from knowledge of the way this gene works from the Thylacine?

MARILYN RENFREE
Well this particular gene we chose because it was small, it?s actually a promoter of the collagen gene. It?s small and highly conserved, so we chose this gene as proof of principle. So this was really the first test, if you like, that we could get the technique to work and indeed it worked very dramatically.
So now it will be possible to select other genes that may be of more interest to us as reproductive and development biologists to investigate their function to see if we can learn something about how the Tasmanian Tiger did things.
The main advance that this study made was to show that you could take a piece of DNA from an extinct individual and put it into a living organism and have it expressed and that was a world first.

SHANE HUNTINGTON
It?s certainly exceptional work. I understand it?s the COL 2 A1 gene?

MARILYN RENFREE
COL 2 A1 promoter.

SHANE HUNTINGTON
Promoter gene.

ANDREW PASK
That?s right.

SHANE HUNTINGTON
Now I?m trying to get a feel for how much of the genome that one gene represents. How much of the puzzle do you have, give or take?

MARILYN RENFREE
Well there are 20,000 genes in a genome and this was one gene. So I guess a very, very small part. Andy, do you want to add something more to that?

ANDREW PASK
Definitely getting more sequences of the Tasmanian Tiger is something that we?re trying to do now. Now there have been a lot of advances in the genetics field in how to sequence entire genomes of species very rapidly and so we?re hoping to invest some of that technology in trying to generate a lot more sequence from the Tasmanian Tiger. So we can start to address some of these more interesting questions that Marilyn spoke about before. So looking at genes now that may be specific to the Tasmanian Tiger. Something that is really unique to that particular animal.

MARILYN RENFREE
Could I just add to that, in fact in Nature, the 20th of November 2008, is the sequence of the mammoth genome. The whole mammoth genome is now published in Nature. And there is a whole article, within it, on speculation about how they?re going to make a mammoth.

SHANE HUNTINGTON
Sounds excellent. You guys were the first to bring back an extinct biological function using this sort of process, correct?

MARILYN RENFREE
Yes.

SHANE HUNTINGTON
Now watching the news broadcast that some people may have had the benefit of around the world, they would have seen this work and the commentary from many of our news broadcasters was that we?d expect to see baby Thylacine?s running around our lounge rooms within a very short space of time as a result of this research.
My understanding is that?s not quite right, is it Andrew?

ANDREW PASK
No, that?s right. Actually bringing back a species from extinction, unfortunately, we?re still a very long way off having the technology to do that. While we can sequence small parts of the genome, even if we had the entire sequence of the genome, we still would have to try and make that into full chromosomes and know how it all fitted together and because the pieces of the puzzle of the Thylacine genome is so small because the DNA is fragmented into tiny pieces, it?s a very, very difficult task.

MARILYN RENFREE
The sequence of steps you would need, would be not only defining the sequence of the genes, but package them up into chromosomes. We don?t know how many chromosomes a Tasmanian Tiger had so we?d have to guess. We could guess from their other relatives.
You?d then have to put these into some sort of nuclear envelope, then you?d put the nucleus into an egg of a suitable recipient species and the suitable recipient species might be the Tasmanian Devil. However, the Tasmanian Devil at the moment has its own problems and is rapidly becoming endangered.
Assuming that you could do all that and get an egg of something like a Tasmanian Devil, then you?d have to put it into the Tasmanian Devil uterus and hope that it would grow and that the conditions would be similar enough that it would support life. Then for a marsupial you would have to assume that the milk was the right composition for the young once it was born. So there are very, very many steps.

SHANE HUNTINGTON
Andrew on this issue, would it even be ethical to bring back a species such as the Thylacine?

ANDREW PASK
I think for a species that we ourselves have made extinct I don?t have an ethical issue, I don?t think, in bringing that back. I mean it was human?s fault that we sent the animal extinct in the first place, so I think we?ve almost ethically obliged ourselves to bring it back if we have the technology to do so.
But I think for other species that have died out for other reasons, like dinosaurs and things - which you can?t get enough DNA from anyway to even start it - probably you should let them lie.

MARILYN RENFREE
I think there would be severe ethical issues if you were to bring back a Neanderthal.

SHANE HUNTINGTON
I?m curious. It?s getting on towards a century now since we?ve seen this particular creature wandering around in Tasmania, how has the rest of the ecology changed as a result of its absence? And could it be reintroduced in a way that wouldn?t cause significant problems on that small island?

MARILYN RENFREE

I think it could be. The ecosystem hasn?t changed that much in Tasmania and the Tasmanian Devil assumed the role of the top carnivore in Tasmania and it became extremely abundant, until it had this facial tumour disease. Perhaps overabundant and so many were killed on the roads, just by cars, every year.
The Tasmanian Tiger - if it was introduced back into Tasmania - would compete to a certain extent with the Tasmanian Devil and would probably keep its numbers more at the sustainable levels than have been allowed to happen without the top carnivore, which was the Tiger.

SHANE HUNTINGTON
Marilyn this is some amazing research spanning the area both of living marsupials and those that have since passed on that we no long have as much access too. I congratulate both you and Andrew on the amazing achievements you?ve had over the last couple of years in these areas.
Professor Marilyn Renfree from the Department of Zoology at the University of Melbourne, and Associate Professor Andrew Pask from Molecular and Cell Biology at the University of Connecticut in the United States, thank you for being our guests on Up Close today.

MARILYN RENFREE
Thank you.

ANDREW PASK
Thank you.

SHANE HUNTINGTON
Relevant links, a full transcript, and more info on this episode can be found on our website at upclose.unimelb.edu.au. We also invite you to leave your comments or feedback on this, or any episode of up close, simply click on the add new comment link at the bottom of the episode page.
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I?m Doctor Shane Huntington, until next time, good bye.

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