

Firstly I would like to say thank you to her Excellency the honourable Kate Warner, Governor of Tasmania and Mr Warner for hosting us tonight in magnificent Government House.

Herzlich Willkommen auf dem Deutschen Botschafter Herr Dr Christoff Mueller. Ich begreuesse auch Frau Dr Katrin Amian von der Alexander von Humboldt Stiftung. Hertlich Willkommen in Australien.

Thank you also to the Australian Association of von Humboldt Fellows especially Dr Peter Jarvis for the warm invitation many months ago now to give this opening lecture and can I say what a privilege it is to be able to talk about this particular subject matter in Tasmania, the home of the Tasmanian tiger, or Thylacine, or as Tasmania's first people knew it, Kanunnah.

Slide.

Tasmania represents a major conservation success story. Many of you will know that as much as 45% of the states land area is protected within reserves, conservation areas, and state and national parks. The first political party in the world dedicated to the environment was born here, the Greens. And, compared to mainland Australia's shocking loss of 30 mammal species since European settlement, Tasmania has only lost a single mammal species.

Slide.

But what an animal to lose. The largest extant marsupial carnivore on the planet and the greatest example of what Darwin referred to as convergent evolution within all of Mammalia! But I will talk more about this later...

Slide.

For those here that are unfamiliar with the tale of the thylacine, its demise was largely heralded by a motion in the Tasmanian Parliament in 1887 (128 years ago), not 2km from our current location here at Government House, which led to the imposition of a bounty payment for captured animals starting in 1888 and finishing in 1909.

Slide.

If you have not yet done so, I would encourage everyone here to read Robert Paddle's book "The Last Tasmanian Tiger", which provides a clinical investigation into the demise of the species and demonstrates clearly that the bounty was a politically motivated tragedy perpetuated by the sheep lobby of the day in response to numerous other factors affecting the agricultural industry at the time. In fact, at the very same time that the Government bounty was introduced, museums and zoos both in Australia and abroad were desperate to acquire increasingly rare specimens.

Slide.

There was opposition when the bounty was introduced. Fittingly for this audience, Robert Paddle chose to quote, in his book, the famous German anthropologist Hermann Klaatsch, who carried out extensive work in Northern Australia in the early 1900's. Among other notable scientists and conservationists Klaatsch remarked at the time that "Tasmania's animals *"were being totally exterminated, with no understanding and with no pity, the hard-hearted and ignorant colonists shooting everything on sight"*.

Slide.

In 1914, Theodore Thomson Flynn, who was awarded the first chair in Biology here in Hobart in 1911, was a fellow of the Royal Society of Tasmania, grandfather of marsupial and monotreme reproductive biology, and father to Errol Flynn, the Hollywood actor, proposed the creation of a thylacine sanctuary to preserve the species. A strategy that is currently employed to protect the Tasmanian devil. However, Paddle writes that "The recognition that it requires both political and direct action to change government attitudes, rather than just the constant repetition of scientifically demonstrable data in professional and occasionally public spheres, grew too slowly to save the thylacine."

Slide.

I'm sure many of you have heard these facts before. From 1888 to 1909 over twenty-two hundred thylacine specimens were presented for a total bounty payment of 2132 pounds and 10 shillings. In 1926, the London Zoo acquired its last specimen for 150 pounds. In 1933, the last known animal was captured in the wild, and on the 7th September 1936 the last captive animal died at the Hobart Zoo. While wild animals almost certainly survived after this time there has been no conclusive evidence of living animals such as a fresh carcass or road kill, or DNA evidence from scats or other material, of which I have personally tested numerous samples. Paddle writes, "It remains a challenge for all modern-day economic rationalists to identify another situation of such significant biodiversity reduction, where so much wanton environmental destruction has been obtained for so little reason and so little money".

Slide.

There are now 754 specimens of thylacines in museums and private collections around the world. These range from jars of formalin fixed stomachs and other organs, complete pouch young as you saw in the first slide from the Tasmanian Museum and Art Gallery, and bones and skins which make up the majority of specimens. The remarkable thing about these specimens is that the majority of them are located outside of Australia, emphasising the extraordinary lengths that foreign institutions and collectors were prepared to go to obtain specimens.

Slide.

So, there is something missing from the Tasmanian bush as a result of our direct actions as a society. There is a collective hole in the psyche of anyone Australian or otherwise who has learned about the thylacine's plight, that represents a lost opportunity to know more about this unique animal. We learned surprisingly little about the thylacine prior to its extinction. How was it related to other marsupials? How did it hunt? What did it eat? What kind of vocalisations did it make? What kind of placenta did it have? How did it evolve to look so much like a dog when it is more closely related to a kangaroo than canines? As I hope to demonstrate, some of these questions may be answerable with the use of modern technology? However, others are not. But, given that humans are directly responsible for the extinction of this unique animal, do we not have an obligation to reasonably discover as much as we can?

Slide.

In 2008, Associate Professor Andrew Pask and colleagues published a paper demonstrating the similar function of an isolated THYLACINE gene that had been

incorporated into a developing mouse embryo. The green-blue staining in the embryo on the left shows that the thylacine gene, which is not that different from the analogous mouse gene, performs the same function in cartilage formation during development. The experiment was a proof of concept that it was at least possible to examine the function of genes from extinct species in living animal models.

Slide.

The findings received broad national and international media attention, but non-scientific audiences tried to understand just what exactly these findings meant. Did this mean they were now close to cloning a thylacine? Were they trying to create thylacine-like mice? I particularly like this cartoon that was published shortly after the 2008 paper was released because I think it crystallizes many of the fears that are associated with research that involves genetic manipulation of embryos. That is, that nutty scientists lacking any oversight are creating Jurassic park style hybrid monsters.

In reality, the majority of projects and publications using ancient DNA are the result of strict adherence to the scientific process and involve rigorous, logical hypotheses, experiments and conclusions. To this end, I am personally interested in experiments that can tell us more about the evolution and ecology of the thylacine. While I am happy to acknowledge that the thylacine may still exist or may one day be cloned, this is not the objective of our research.

Slide.

To understand the questions we seek to answer by studying thylacine DNA it is important to understand the

prehistoric origin of mammals in Australia. Importantly, Australia is the best place in the world to study mammalian diversity because we are the only country besides New Guinea and Indonesia to have representatives of all three extant groups including the egg laying monotremes which are the oldest living mammals having last shared a common ancestor with humans up to 200 millions years ago. This estimate is based on molecular dating because no fossil monotremes older than 100mya have ever been found. In contrast, the live-bearing marsupial mammals share a more recent ancestry with humans, which are eutherian mammals. Fossils from China show that a distinct eutherian mammal, was already present 160 million years ago. This evolutionary distance between eutherian and marsupial mammals is very helpful for understanding genome evolution as I will discuss shortly.

Slide.

While marsupials were known to the indigenous inhabitants of Australasia and South America for many thousands of years they only came to the attention of western explorers in 1501 when Vincente Pinzon captured a female North American opossum, which he later presented to the King and Queen of Spain in Grenada. Following this there was a brief description of a cuscus from a Portuguese office stationed in the spice islands sometime in the 1530s...he noted that "On their belly they have a pocket like an intermediate balcony; as soon as they give birth to a young one they grow it inside there at a nipple until it does not need nursing any more"

Slide.

It is not until 1629, during the ill-fated shipwreck and mutiny of the Dutch ship Batavia in the Abrolhos

archipelago off the coast of Geraldton, WA, that the first description of an Australian marsupial occurred. The mutiny of the Batavia remains to this day as one of the worst maritime disasters in history during which 110 men, women and children were murdered by the mutineers in attempt to ambush Commander Francisco Paelsart when he returned with help and supplies from Java.

Slide.

Amongst this madness, Paelsart penned the first description of the tammar wallaby. He notes that *"We found in these islands large numbers of a species of cats, which are very strange creatures. It has two hind-legs and it walks on these only. Its tail is very long; if it eats, it sits on its hind legs, and clutches its food with its forepaws."* Remarkably, the first Australian marsupial discovered by the western world, was also the first to have its genome sequenced. WHY IS THIS? Put simply, among marsupials, the tammar wallaby is bomb proof. In the Houtmann Abrolhos where it was discovered by Paelsart, the tammar lives on saltbush and does not need to drink fresh water, which is lucky because most of the time there is none there. For these reasons it is very easy to maintain in captivity and has remained a model species for the study of marsupial reproduction for the last 50 years.

Slide.

Paelsart also noted the unique mode of reproduction that is common to all marsupials, and distinguishes them from their eutherian cousins. He writes that, *"Their manner of generation or procreation is exceedingly strange. Below the belly the female carries a pouch and we have found that the young ones grow up in this pouch until they are able to walk..."*

Slide.

The three subclasses of mammals, monotremes, marsupials and eutherians differ most markedly in their manner of reproduction and much of Flynn's pioneering work was to describe the early embryology of marsupial and monotreme embryos for which he was awarded a doctor of science in 1921. Flynn was also instrumental in dispelling the myth that marsupials were born out of the nipple. Importantly, while all marsupial embryos possess a stretchable outer shell coat during development, this is lost during the last third of pregnancy during which they form a fully functional placenta similar to eutherian species.

Slide.

I recently returned from Berlin where I was collaborating on another project with my Humboldt partner Professor Thomas Hildebrandt. Our subsequent studies are the first to examine marsupial reproduction by ultrasound. Here you can see an image of an embryo that Flynn and others would have had to dissect from a dead animal. The colour Doppler around the outside of the embryo shows the tremendous blood flow through the endometrium, like a solar eclipse, as it delivers nutrients to the expanding wallaby vesicle.

Slide.

Here is the tammar wallaby embryo before birth. Here you can see the fetal head including the mouth and developing eye. A small forearm here, and the all-important placenta. Amazingly, fetal wallabies practise their crawl to the pouch before birth with alternating movements of their two

forearms, which is possibly the earliest behaviour observed in any mammal.

Slide.

The thylacine also gave birth to tiny young, weighing less than a gram, that crawled into its backward facing pouch where they would have likely developed for 5-6 months. Thylacine birth was never observed so it is not known if, like the Tasmanian devil, they gave birth to excess young that then raced to the pouch seeking limited teats. However, they were often observed with 3 or 4 cubs as seen in this photo here.

Slide.

It is still unclear why marsupials persisted in Australasia while eutherian species did not. Australia, being the oldest continent on earth, has relatively few deposits of fossil mammals. However, it is clear that primitive forms of many of the major families including the thylacine, kangaroos, marsupial mice, marsupial moles and others were already present by the late oligocene (20-25 mya). In fact it wasn't until approximately 10-15 million years ago that bats arrived in Australia, 5-10 mya for rodents and only a few thousand years for the dingo. All of these eutherian late-comers likely reached Australia via the Indonesian archipelago.

Slide.

Up to 7 different species of thylacine of various sizes have been identified from fossil deposits on mainland Australia. However, it was only *Thylacinus cynocephalus* that persisted until both Indigenous and European humans arrived in Australia, respectively. Approximately 10-13 thousand years ago the flooding of Bass Strait separated

the mainland and Tasmanian populations of thylacines. 3-5 thousand years ago the Dingo arrived on the mainland and the thylacine became extinct there about 1-2 thousand years ago, with only the Tasmanian population remaining.

Slide.

During my Humboldt fellowship from 2010-2012 I sampled a number of thylacine specimens from museums in Germany, Britain and Australia to understand more about the genetic health of the species by comparing the variability of their mitochondrial DNA. Our study estimated that there are on average only 8 nucleotide differences between the mitochondrial genomes of individual thylacines. This compares with a figure of 10 in the Tasmanian devil, 77 in wolves and 22 in Europeans. This lack of genetic diversity is a reflection of the relative ability of populations to fight disease and to cope with changing environments and it appears that many Tasmanian mammal populations have similarly low genetic diversity.

Slide.

The reason I referred to some marsupial species earlier as marsupial mice or marsupial moles is that many marsupials bear a striking resemblance to their eutherian counterparts in other parts of the world due to a process known as convergent evolution. The small, rodent-like marsupial ancestor of all the marsupials, evolved in isolation in Australasia. Remarkably, 50 million years of environmental selection produced many of the same anatomical outcomes seen in eutherian species around the world including marsupials that glide through the forest like eutherian squirrels and of course, a marsupial version of a canine, wolf, or dog.

Slide.

The thylacine and dog are so similar that the only anatomical characteristics to tell them apart was the presence of a pouch in female specimens and the posterior positioning of the penis relative to the testes in male specimens, the latter a hallmark of all marsupials. The only obvious differences in the skulls are the two large holes in the maxillae or upper jaw of the thylacine compared to the dog.

Slide.

A fundamental question in evolution is how natural selection works at the level of the gene or genome to gradually drive the dramatic anatomical differences observed between species of similar ancestry. Here you can see the analogous bones of the skeletal forelimbs of the bat and mouse side by side. We know that evolution has selected for these differences over time somehow, but we don't know for example if this was due to say changes in the protein coding portion of specific genes, or changes to the myriad regulatory sequences that affect gene expression, or the production of certain proteins.

Slide.

The thylacine genome, or, the sum of all of the DNA sequences that existed within a single thylacine cell, could provide a natural template for understanding the aforementioned selective processes.

Slide.

If the thylacine and the dog obtained a similar anatomical design via the same changes to their DNA then we should be able to identify those regions of the genome that are

more similar between dog and thylacine, than between say the thylacine and the wallaby, a more closely related species, but one that is very different anatomically.

Slide.

Thus, a few years ago, Andrew Pask, myself and others began testing the feasibility of sequencing the thylacine genome by testing a variety of tissue samples that we had collected from previous experiments on genetic diversity and gene function. These samples are precious and finite, so we wanted to examine the quality of DNA first before committing them to genome sequencing. One organ sample from a museum victoria specimen preserved in alcohol, pictured here, was of particularly good quality.

Slide.

We then carried out a test run of this sample using next generation sequencing machines and looked for contamination of the sample with foreign DNA from bacterial or human sources by comparing the resulting sequences with other genome databases. The sample had minimal contamination with the majority of sequences showing similarity to other marsupial species, which we would expect given the thylacine is a marsupial.

Slide.

So, we proceeded with a full scale sequencing effort with this sample using a combination of gene sequencing platforms, and it gives me great pleasure to introduce you today to the first build of the Thylacine Genome 1.0. The total size of the thylacine genome is approximately 3 Gigabases or 3 billion bases, which is of similar size to other marsupials including the tammar wallaby. The genome currently consists of just over 2 million units

known as contigs or contiguous blocks of overlapping DNA segments. This is somewhat higher than genomes from extant species and reflects the fragmented nature of much of the DNA. Even so, the average lengths of these contiguous units of DNA are 3,500 base pairs in length. The longest contiguous sequence of DNA assembled was nearly 40,000 base pairs in length. To put this in perspective, the mitochondrial genome of the thylacine published in 2009 was about 15,000 base pairs. Our sequencing efforts to date have produced a 15x high quality coverage genome, which means that on average, each base pair of DNA in the genome has been sequenced 15x, making it the most complete genome of an extinct animal to date.

Slide.

If the contiguous DNA segments for the thylacine are mapped against other marsupial genomes you can see that the sequences are most similar to the Tasmanian devil, a close relative, but less similar to more distantly related marsupials such as the wallaby and south-American opossum, which we would expect.

Slide.

The genome sequence is also useful for understanding relationships between other carnivorous or insectivorous marsupials. This is important as three previous molecular studies have published 3 different results in respect the phylogenetic placement of the thylacine.

Slide.

We have used a method known as retroposon tracking to unequivocally determine the phylogeny of the thylacine. This method was used recently to show that the South

American mammal Dromiciops (known locally as Monito del Monte) is more closely related to the Australian marsupials than those in South America, making it the most basal Australidelphid. This method is based on the random incorporation of DNA from retroviruses into the genome of mammals over vast distances of evolutionary time. Because the viral DNA is non-functional it remains as a permanent feature of the genome of all the descendants of that species. By counting the number of insertions between species, indicated by the white dots in this image, it is possible to determine precise genetic relationships. For example dromiciops here has all of the insertions along its evolutionary timeline from a south american ancestor, but it does not have those ones specific to the other south american marsupials that diverged earlier in evolutionary time. Similarly, they do not have those viral insertions acquired by dromiciops later in evolutionary time.

Slide

In summary, I hope I have shown that marsupials provide fantastic models for research and we should encourage young scientists to continue to use them in the future and to ask bold scientific questions. We have produced a high quality genome from an iconic, extinct species that can now be used to ask a range of questions about diet, ecology and phylogeny of the thylacine.

To conclude, the tale of the thylacine provides a necessary lesson to society about the importance of gaining many perspectives, a global perspective if you will, when making decisions concerning our unique environment. It has truly taken a global effort to collect, sequence and assemble dozens of thylacine samples so that we may truly appreciate this unique animal that was so tragically lost to history. In fact, by bringing the

thylacines DNA to life we may in the future be able to gain fundamental insight as to how evolution works at the molecular scale.

Thank you and particular thanks to our many collaborators including Andrew Pask who has been a major driving force behind our continued interest in everything thylacine.