



Some Australian marsupials bear striking resemblances with South American species, but is this because they are extremely close cousins, or have they adapted independently to living in similar environments? Their DNA might have the answer. Credit: Phil Spark

What do genomes have to say about marsupial history?

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Marsupials have walked on Australia for tens of millions of years, and include iconic species like the kangaroo, wombat and bilby. New methods for studying the genomes of marsupials have revealed some surprising aspects of their evolution.

When humans first walked the red sands of Australia more than 50,000 years ago, they were probably mesmerised by the unique mammals that inhabited every corner of the continent: the marsupials.

It is easy to become fond of this remarkable group of mammals, and understandably they have inspired stories for thousands of years. In addition to being a crucial part of Australian identity and culture, marsupials are fascinating to the scientific community.

The Ancestors of Marsupials

Marsupials are a group of around 334 living species with a rich history. Their ancestors experienced several major changes in the world. Australia was inhabited by marsupials even before

it was a separate landmass. Early marsupials lived on a mega-continent called Gondwana, a land in which Australia was joined with what we now call Antarctica and South America. At this point in the dawn of marsupial history, they had recently become distinct from their closest living relatives, the placental mammals.

The world has undergone enormous changes since those times around 70 million years ago. It was a time when Gondwana was covered in thick and warm rainforests and was roamed by dinosaurs. To imagine Australia fully covered in wet forest is in stark contrast to the red sands and grasslands that now dominate much of the continent.

The fact that marsupials existed at a time when Australia was connected with several other continents explains why around 30% of species are now found in the Americas. The

marsupials from the two continents are close relatives and have astounding resemblances. Most obvious is the presence of a marsupium, derived from the Ancient Greek word for “pouch”. This is a place in the body where marsupials spend the latter stages of their embryonic development, and then some time afterwards while feeding on their mother’s milk.

But the similarities between American and Australian marsupials go much further. One might struggle to tell the difference between the South American monito del monte and the Australian pygmy possum. The similarities between some Australian and American marsupials are possibly because they inhabited the same types of environments after the continents split. Hence the two groups of marsupials might have developed similar adaptations over millions of years, such as adaptations that helped them live in the forest canopy.

Alternatively, marsupials might look similar across continents not due to adaptations but because they inherited the same set of features from their common ancestors. This could have substantial implications for our understanding of their history. It could be that Gondwana had already accumulated a great diversity of marsupials so its break-up caused the splitting of close relatives between Australia and South America. Some American species, like the monito del monte, would then be more related to Australian species than to their American neighbours.

DNA Clues to Marsupial History

So what is the reason for the similarity between such geographically distant marsupials? Did they adapt to similar types of environments, or are they very close relatives?

Many researchers have carefully investigated the physical resemblances between species. If any two species look very similar it could be because they are close relatives. Comparing physical appearance between animals is often extremely useful, but it can also be misleading. Species that look similar are not necessarily closely related, while those that are very different might have simply evolved novel features over a short space of time.

An alternative to investigating the resemblance in physical appearance is to look much deeper into the primary materials that build an animal. By sequencing the DNA in their genome it is possible to get an exact picture of the similarities and ancestry between animals.

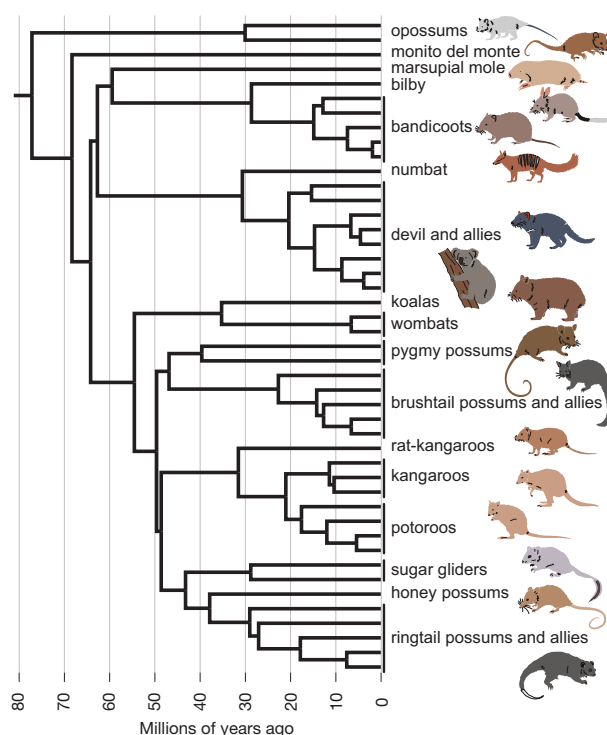
One of the reasons genomes are so useful is that they have a vast amount of information that can be compared. If you think of an instruction manual divided into chapters, phrases,



words and letters, the genome is also divided into chromosomes, genes, gene regions and nucleotides. Every letter, or nucleotide, can be compared between species, and the genome of a kangaroo has billions of these letters you can use for comparison with other marsupials.

In a recent study, I worked with colleagues from several institutions to extract the sequence of DNA nucleotides and compare them between marsupial species. We were particularly interested in the differences between species to understand their relationships to one another.

The relationships between marsupials and the timing of their evolution as described using thousands of sections of their genomes.





The monito del monte is native only to Argentina and Chile, yet it is more closely related to Australian marsupials than to most other American mammals. Credit: José Luis Bartheld

Studying the genome has greatly improved our understanding of marsupials. For example, it turns out that the South American monito del monte is not quite a pygmy possum, despite their great resemblance. However, it is indeed more closely related to Australian marsupials than to its South American neighbours.

Another question that has been resolved by comparing genomes is where the Australian marsupial mole fits into the marsupial family tree. This enigmatic creature lives underground in remote Australian deserts, hunting for insects and small reptiles. It was once thought to be a close relative of the placental mole rather than being a marsupial at all.

Past studies using small chunks of the genome had great difficulty reaching definitive results, and studies that focused on physical features would suggest that it looked more like a bandicoot. Surely enough, when we compared large swathes of the genome across species, we found convincing evidence that the closest living relatives of the marsupial mole are the bandicoots.

But genomes are not the be-all and end-all, and sometimes we need help from other sources of information, like the fossil remains of long-extinct animals. For example, the desert-living marsupial moles have existed for a very long time, so without any additional information we might imagine that they have lived in the desert throughout their entire history.

However, this would ignore the fact that most of the desert in Australia is only young (around 10 million years) compared with the lineage of marsupial moles (more than 50 million years). Fossils have indeed shown that the ancestors of the marsupial mole were adapted to living in the rainforest. This demonstrates the importance of the fossil record when trying to draw a complete picture of the evolution of marsupials.

Our team also stumbled upon the fact that genomes can be

difficult monsters to tame. Being large, one part of the genome can be very different from another. We were particularly struck by this fact when trying to solve the relationships between the different types of possums (ringtails, brushtails, honey possums and the like) and the kangaroos. Are all these possums equally closely related to the kangaroos, or are some possums more closely related to kangaroos than others?

We devised a novel technique to examine what different parts of the genome said about the similarities between marsupials. Strikingly, the genome was split into two in terms of its information. One part of the genome indicated that brushtail possums and kangaroos are close relatives, while the ringtail possums are less closely related. The other, even larger part of the genome gave a contradictory message: that it was the ringtail possums that were most closely related to the kangaroos! Further examination helped us solve this issue, and led to greater confidence in the latter results: brushtail possums are less related to a group joining the ringtail possums and kangaroos.

Such a split in the information is consistent with previous studies that have shown that marsupial genomes can be peculiar. In rock wallabies, large sections of genomes have split, joined, jumped and become inverted repeatedly in what is known as chromosome rearrangements. This kind of genomic gymnastics is a powerful driver of novelty, and might even be the reason why species of rock wallabies have become different to each other.

Sequencing genomes has proven to be a powerful tool for resolving the relationships of different species across the tree of life. Plans are underway to sequence the genomes of every species of Australian mammal, including the marsupials. This provides an exciting opportunity to discover how their evolution has been shaped by the flora and environment of Australia through time.

Some insights have already been found regarding how environmental changes shaped marsupials. For example, the expansion of grasslands provided a prime environment for kangaroos to diversify.

Meanwhile, fossil data will be more relevant than ever to understand the drivers of marsupial diversification. Fossils might help explain why there used to be giant marsupials, like the hippo-sized but wombat-looking *Diprotodon*, and whether humans might have contributed to the extinction of some of these creatures.

Research with large amounts of data from the genome and fossils holds great promise to reveal the history of marsupials. Creative approaches to looking at these data will also be necessary for understanding how marsupials are related, how they interacted with their changing environment, and why their genomes, like them, are so very peculiar.

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