REWRITING EVOLUTION

Tiny molecules called microRNAs are tearing apart traditional ideas about the animal family tree.

BY ELIE DOLGIN

Kevin Peterson grabs a pen and starts to scribble an evolutionary tree on the paper tablecloth of a bar in Hanover, New Hampshire. Drawing upside down to make it easier for me to see, he maps out the standard phylogenetic tale for placental mammals. First, Peterson scratches a line leading to elephants, which branched away from the rest of the placentals around 90 million years ago. Then came dogs, followed by primates (including humans) and finally rodents — all within a frenetic 20 million years. This family tree is backed up by reams of genomic and morphological data, and is well accepted by the palaeontological community. Yet, says Peterson, the tree is all wrong.

A molecular palaeobiologist at nearby Dartmouth College, Peterson has been reshaping phylogenetic trees for the past few years, ever since he pioneered a technique that uses short molecules called microRNAs to work out evolutionary branchings. He has now sketched out a radically different diagram for mammals: one that aligns humans more closely with elephants than with rodents.

"I've looked at thousands of microRNA genes, and I can't find a single example that would support the traditional tree," he says. The technique "just changes everything about our understanding of mammal evolution".

Peterson didn't set out to rewrite textbooks. A mild-mannered but straight-talking Montanan, Peterson had made a quiet career studying how bilateral body plans originated more than 500 million years ago. He has a particular interest in marine invertebrates and had intended to stick with that relatively obscure branch of the animal tree. But a chance investigation of microRNAs in microscopic creatures called rotifers led him to examine these regulatory molecules in everything from insects to sea urchins. And as he continues to look, he keeps uncovering problems, from the base of the animal tree all the way up to its crown.

That has won him many critics, but also some strong supporters. "Peterson and his colleagues have demonstrated that microRNAs are a powerful tool in determining the relationships of major animal groups," says Derek Briggs, director of the Yale Peabody Museum of Natural History in New Haven, Connecticut.

Now, together with his colleagues around the world, Peterson is putting it all on the line with mammals. "If we get this wrong, all faith that anyone has in microRNAs [for phylogenetics] will be lost," says Philip Donoghue, a palaeobiologist at the University of Bristol, UK, who has teamed up with Peterson. And there is more at stake than just the technique. "It could well be the end of all our careers," he says.

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FOSSIL FIND

If Peterson does end up switching careers, it won't be the first time. In the early 1990s, he was working the night shift unloading trucks at a freight company in his hometown of Helena, Montana, trying to figure out what to do with his life. He had recently graduated with a pre-medical degree from a local liberal arts college, but he knew he didn't want to become a doctor. Then, rummaging in his parents' barn, he happened on the first fossil he had ever collected, as a four-year-old: a crinoid, or sea lily, about the size of a button. "After I found it, I knew right away that this was what I wanted to do," he says. "I applied to graduate school the next week."

He soon enrolled in a PhD programme in the Department of Earth and Space Sciences at the University of California, Los Angeles. There, he teamed up with developmental geneticists Eric Davidson and Andrew Cameron at the California Institute of Technology in Pasadena, and over the course of his

Kevin Peterson has kicked many mammals, including the Alaskan Brown Bear, off their traditional perch on the evolutionary tree.

DANA SMITH

graduate and postdoctoral work the three men developed a provocative idea, dubbed the set-aside cell hypothesis[1]. They posited that the ancestor of modern-day animals was a larva-like creature containing a group of undifferentiated cells that retained the capacity to give rise to the spectrum of adult body types seen during the Cambrian explosion. The idea subsequently came under fire from the evolutionary and developmental-biology communities.

A few years after moving to Dartmouth in 2000 to start his own lab, Peterson was looking for a way to test the hypothesis when he became intrigued with microRNAs. First discovered in 1993 by Victor Ambros, now at the University of Massachusetts Medical School in Worcester, these short, hairpin-shaped molecules bind to messenger RNAs and stop them from making proteins. A team that included Davidson had shown that a microRNA called let-7 was present in animal lineages that had bilateral body plans but not in simpler organisms such as jellyfish and sponges[2], hinting that microRNAs could hold the secret to morphological complexity.

Peterson teamed up with Lorenzo Sempere, then a graduate student working with Ambros at Dartmouth, and the pair began to search for let-7 and a handful of other microRNAs in relatively simple invertebrates, including rotifers, and in more complex creatures. As they added more microRNAs, they found a clear pattern: the farther away from the trunk of the evolutionary tree the animals were, the more microRNAs they had accumulated[3]. The pair started to realize that the molecules provided "a brand new way to do phylogeny, using a set of rare genomic characters that no one had ever considered before", Peterson says.

"IT COULD WELL BE THE END OF ALL OUR CAREERS."

MicroRNAs, Peterson and Sempere discovered, are unlike any of the other molecular metrics that biologists typically use to tease apart evolutionary relationships. DNA binding sites, for example, continuously mutate; microRNAs, by contrast, are either there or they aren't, so their interpretation doesn't require such complex sequence and alignment analyses. And once gained, microRNAs usually remain functional, which means that their signal stays intact for hundreds of millions of years. "No gene family was known to evolve in this way," Peterson says. In addition, these small molecules are often expressed in specific tissues and help to regulate the development of certain organs, so they could explain the origin of morphological innovations over geological time[4].

According to Peterson's latest tally, 778 microRNA families have arisen during the 600 million or so years of animal evolution, and only 48 have been lost. This pattern of inheritance leaves an easy-to-follow evolutionary trail for phylogenetic sleuths. Eugene Berezikov, a geneticist who studies microRNAs at the Hubrecht Institute in Utrecht, the Netherlands, says that microRNAs give a clearer answer than other molecular markers of evolution "because the analysis is much simpler".

OUT OF OBSCURITY

At first, Peterson and Sempere had a tough time publishing their results suggesting that animals had accumulated regulatory microRNAs. "One of the reviewers said it was impossible, what we were describing," says Peterson. In the end, the work was published in a specialized zoology journal[3]. Subsequent papers, however, won over some sceptics and Peterson was soon publishing in *Nature* and *Science*, and using his growing microRNA

library to resolve relationships within and between an assortment of evolutionary lineages, from jawless fishes[5] and reptiles[6] to fruitflies[7] and acoelomorph worms[8].

"It is a really clever and fresh approach to phylogeny," says Peter Stadler, an evolutionary bioinformatician at the University of Leipzig in Germany. "I don't quite know why presence/absence of microRNAs is not used more frequently in deep phylogeny approaches."

Still, not everyone is convinced that microRNA genes trump other types of phylogenetic data. A key point of contention is whether microRNAs only rarely drop out of the genome, as Peterson contends. Andreas Hejnol, who studies invertebrate evolution at the Sars International Centre for Marine Molecular Biology in Bergen, Norway, is sceptical. "MicroRNAs behave like other genes — namely, they can be lost," he says. "There's no special mystery about them." Travis Glenn, an evolutionary biologist at the University of Georgia in Athens, agrees, saying that microRNA losses are probably underestimated. In May, he and his colleagues published a retort[9] to a paper[6] in which Peterson had argued that turtles are more closely related to lizards than to birds and crocodiles — the opposite of what most genomic data sets had indicated. Glenn argued that ultraconserved DNA elements — ones that evolution has kept intact over a long time — show that the conventional view is correct.

The critics have mostly been a vocal minority, but as Peterson climbs up the evolutionary ladder with his microRNA analyses, he will be reaching a much bigger audience — and the detractors are likely to become a lot louder. "We're mammals, so this matters," he says.

UP A TREE

When Peterson started his work on the placental phylogeny, he had originally intended to validate the traditional mammal tree, not chop it down. As he was experimenting with his growing microRNA library, he applied it to mammals because their tree was so well established that they seemed an ideal test. Alas, the data didn't cooperate. If the traditional tree was correct, then an unprecedented number of microRNA genes would have to have been lost, and Peterson considers that highly unlikely. "The microRNAs are totally unambiguous," he says, "but they give a totally different tree from what everyone else wants."

The results change the image of the proto-placental mammal. Because microRNAs place mice and rats at the base of the placental tree, they suggest that rodent-like traits, such as continuously growing incisor teeth, were common in the first placentals, then lost in the lineage that leads to primates, elephants, dogs and cows (see 'Duelling trees'). The findings also shift the geographical origin of placental mammals, suggesting that they started in the Northern Hemisphere, where the first rodent fossils are found, not in the Southern Hemisphere, as many researchers have assumed on the basis of fossil and DNA data.

At first, Peterson was shocked by his results, which still haven't been published. But he has spent the past year validating his tree with gene-expression libraries and genomic sequences, all of which he says support his findings.

Many supporters of the traditional tree suspect that something peculiar is happening with the microRNAs — probably large losses in the mammalian lineage. "He's talking about the entire genome that has to be wrong," says Robert Asher, a mammalian palaeontologist at the University of Cambridge, UK. "I don't give it any serious consideration," says Mark Springer, a molecular phylogeneticist at the University of California, Riverside, who last year published the most comprehensive genomic data set so far in support of the traditional mammalian tree[10]. "There have to be other explanations," he says.

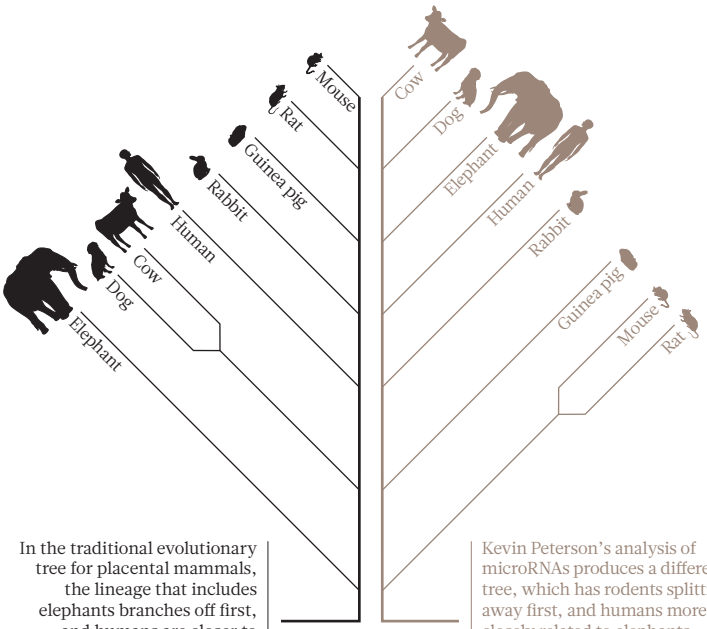
Peterson and his team are now going back to mammalian genomes to investigate why DNA and microRNAs give such different evolutionary trajectories. "What we know at this stage is that we do have a very serious incongruence," says Davide Pisani, a phylogeneticist at the National University of Ireland in Maynooth, who is collaborating on the project. "It looks like either the mammal microRNAs evolved in a totally different way or the traditional topology is wrong. We don't know yet."

Hoping to resolve the issue, Donoghue and phylogeneticist Ziheng Yang at University College London have spent the past year amassing DNA sequences that span more than 14,600 genes from 36 mammalian species — a data set that dwarfs the one used by Springer. They are trying to determine whether the larger crop of DNA data produces the same tree as microRNAs yield. They have been able to date the origin and diversification of placental mammals[11], but they are still working to resolve which lineages branched off first — a key test for the phylogenies.

Peterson would like to put it all behind him. "What sucks about this mammal project is that it's all-consuming," he says. "Ultimately, I don't really care how mammals are related to one another — it doesn't matter to me. But what does matter is the validity of the data set."

If it turns out that the traditional mammal tree is right, Peterson won't see that result as a defeat for microRNAs. It would just mean that something odd happened with mammalian microRNAs, he says. "That says something really interesting about the evolution of microRNAs and the construction of gene regulatory networks in mammalian evolution."

For now, he's trying to amass the best evidence he can before publishing the mammal study. Then he wants to return to the quiet life of an ancient-invertebrate biologist. But if Peterson's voyage upends the mammalian phylogeny, he'll have left a furry mess in his wake. ■



In the traditional evolutionary tree for placental mammals, the lineage that includes elephants branches off first, and humans are closer to rabbits and rodents.

DUELLING TREES

Kevin Peterson's analysis of microRNAs produces a different tree, which has rodents splitting away first, and humans more closely related to elephants, dogs and cows.

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