PALAEOANTHROPOLOGY

Lessons from our cousins

Studies of Neanderthal brain development could provide insights into the evolution and inner workings of the human brain.

BY SEDEER EL-SHOWK

In Petri dishes at the University of California, San Diego, unassuming white clumps of cells grow into miniature replicas of the developing brain. They are brain organoids — simplified models of the brain consisting of hundreds of thousands to several million cells. Although tiny compared with even the fetal brain, and lacking blood vessels and certain cell types, brain organoids created from human cells have already proved valuable. For instance, during the 2015 outbreak of Zika virus in Brazil, researchers used brain organoids to help to show that the virus caused microcephaly[1], a smaller than normal head size in children whose mothers had caught Zika during pregnancy. But the organoids growing in Alysson Muotri's laboratory are different from those that have come before. In his quest to understand the brain's evolution, Muotri engineered brain organoids to carry a variant of a gene that is found in our closest extinct kin — Neanderthals.

Since the publication of the draft Neanderthal genome[2] in 2010, researchers have known that Neanderthals interbred with anatomically modern humans. "Ten years ago, I was teaching that there was little or no inbreeding, and now we know that it was quite frequent," says Philipp Gunz, a palaeoanthropologist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Scientists can now track Neanderthal gene variants in modern humans and, in combination with the analysis of preserved remains, pinpoint genes and developmental processes that have changed in humans since the two species diverged. The effect of these genetic changes can be tested in brain organoids such as those in Muotri's lab. As close relatives, Neanderthals offer an unequalled opportunity to uncover how modern humans probably evolved.

THE SHAPE OF THINGS TO COME

Researchers cannot study the brains of Neanderthals directly — the species is thought to have been extinct for around 40,000 years, and soft tissue doesn't fossilize well. But from preserved skulls, scientists have been able to infer that adult Neanderthals had brains of a similar volume to those of modern humans, but with a more elongated, less globular shape. Finding out when this anatomical difference becomes apparent during brain development could provide a clue as to which aspects of brain development are unique to humans. "We're trying to find out what evolved last," says Gunz. "What is the most recent innovation in our brain?"

To determine the stage in development at which the brains of modern humans and Neanderthals begin to diverge, Gunz and his team used a technique that he established as a postgraduate student to analyse digitally rendered casts of the interior of the skulls, or braincases, of newborn, infant and adult Neanderthals, as well as those of modern humans of European ancestry, created from computed tomography scans. Because neuron formation in the brain is largely complete by birth, knowing the age from which skull shape begins to differ in the two species should reveal whether the globular shape of modern human brains is mainly the result of prenatal neurogenesis or changes in networking and connectivity that occur predominantly after birth.

Anatomically modern humans can be distinguished from Neanderthals (left) by their less elongated, globular skull (right).

PHILIPP GUNZ (CC BY-NC-ND 4.0)

Gunz's analysis suggested that the braincase of a newborn Neanderthal is similar in shape to that of a newborn baby[3]. "That tells us that most of the differences in brain shape developed after birth," says Gunz. He and his team propose that this postnatal growth pattern, which he calls globularization, is unique to the brain development of modern humans and was the most recent feature to evolve.

But earlier analyses tell a different story. Marcia Ponce de León, a palaeoanthropologist at the University of Zurich in Switzerland, says that biases in the reconstruction methods used by Gunz make unfamiliar structures, such as newborn Neanderthal skulls, more likely to resemble known ones, such as modern-human skulls. Using an alternative method, she and her team found differences between newborn Neanderthal and modern human skulls[4], and therefore suggest that the globular shape of the modern-human brain is already present at birth.

Ponce de León suggests that the postnatal changes described by Gunz as globularization

occur in all great apes except chimpanzees. This raises questions about the validity of the chimpanzee brain as a model, as well as which elements of brain development are unique to modern humans. "Many features of the human brain and its development have deep evolutionary roots and are shared with Neanderthals," says Ponce de León. "Humans are not as exceptional as they perceive themselves to be."

THE CROSS OF CHANGES

Regardless of when the differences develop, the distinct braincases of adult modern humans and Neanderthals offer a tool with which to tease out genes involved in brain development. By combining data on these anatomical differences with information from Neanderthal genetic fragments that are scattered throughout the genomes of people of non-African descent, researchers can find brain-related genes that changed after the two species diverged, and can therefore distinguish modern humans from Neanderthals. "Studying the effects of Neanderthal gene variants in modern people is a powerful model system," explains Chet Sherwood, an anthropologist working on evolutionary neuroscience at the George Washington University in Washington DC. "Our genetic background and overall biology resembles them more than other possible models."

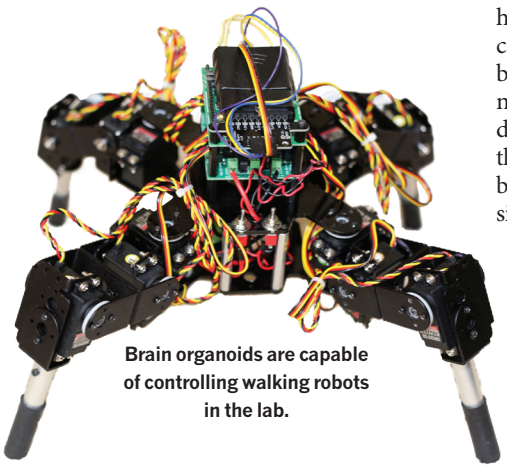
The trick is to identify Neanderthal gene fragments that correlate with a difference in anatomy, such as braincase shape. This reveals genes that influence the trait. Gunz started by studying magnetic resonance images of the brains of modern Europeans, which vary in their globularity. "It's a very subtle shift: nothing you would notice," he says. "There's nobody alive today that has a brain as elongated as a Neanderthal's." Next, Gunz created a score to quantify the globularity of each scanned brain. He then identified gene variants that differed with the score, which revealed brain-related genes that would probably have differed between Neanderthals and modern humans.

The technique led Gunz and his team to two genes in mice, one known to affect neurogenesis in the putamen and another that influences myelination in the cerebellum — both regions of the brain involved in movement. Neither gene had previously been linked to brain evolution. The differences between the modern human and Neanderthal versions of these genes are likely to alter their expression. Of course, these genes are just part of the picture — Gunz stresses that they probably play only a small part in brain development. "We're not trying to argue that these are the two genes that turn you into a modern human," he says. Researchers in the United States are also using the Neanderthal heritage of Europeans to investigate the modern-human brain. They tested whether the overall amount of Neanderthal DNA that a person carries is correlated to their skull shape[5], and found not only that it is, but also that it is correlated to the morphology of both the brain's visual cortex and the intraparietal sulcus — a brain region with roles in visual attention and motor coordination.

Gunz and his colleagues are working to replicate their findings in a larger data set from the UK Biobank repository, which might also enable them to identify other interesting genes. Long-term, the challenge is to discover the precise function of such genes and the effects of their Neanderthal variants. "This is the exciting start of a new research avenue, or probably several avenues heading off in multiple directions," Gunz says.

THE GHOST IN THE BRAIN

The genes that Gunz uncovered act too late in brain development to be studied in brain organoids, which recapitulate only the early stages. But, equipped with the gene-editing tool CRISPR–Cas9 and a copy of the Neanderthal genome, researchers are using brain organoids to study other genes that changed during the evolution of modern humans. The strategy is akin to intentionally substituting an ingredient in a recipe: the outcome of adding a Neanderthal variant of a gene to an otherwise modern-human recipe can teach us about the contribution of the original gene.



MUOTRI LAB/UC SAN DIEGO

Brain organoids are capable of controlling walking robots in the lab.

Muotri is using this approach to study a gene involved in RNA splicing that is strongly expressed by neurons and has been associated with schizophrenia. He wants to understand the evolution of the 'social brain', which is thought to enable people to function in large societies. Comparing modern-human brains with those of a species that led a contrasting life could provide clues, and "there is plenty of evidence suggesting that Neanderthals might have had different life styles and social interactions", Muotri says. In the absence of living Neanderthals, his lab has used genome editing to create 'Neanderoids' — brain organoids containing Neanderthal versions of certain genes.

His team has uncovered intriguing differences, although so far has only presented them at conferences. Brain organoids are typically smooth spheres, but Neanderoids "have a popcorn shape", Muotri says. The researchers have also noted changes in cell proliferation and migration, the formation of neural connections and downstream gene expression, as well as in RNA splicing. This reveals not only the pathways that are influenced by the edited gene, but also how the Neanderthal and modern-human versions differ in their effects.

Muotri is imbuing Neanderoids with other genes to further probe the evolutionary changes that set apart modern-human and Neanderthal brains, and similar work is being done elsewhere, such as in Svante Pääbo's lab at the Max Planck Institute for Evolutionary Anthropology, where the team sequenced the draft Neanderthal genome. Alex Pollen, a neurobiologist at the University of California, San Francisco, who works with chimpanzee brain organoids, supports such efforts. "We can now begin to ask these questions about human origins from a genetic and developmental perspective," he says, because these organoids offer a more faithful environment in which to test modern-human-specific genetic changes than do animal models.

Muotri says that his brain organoids and Neanderoids show a level of activity similar to that observed in the brains of developing humans. He thinks that it might be possible create devices that can provide feedback to the brain organoids that will help to refine their neural networks, similar to what happens during human neurodevelopment. To test the theory, his team is using electrodes to record the brain organoids' activity, and then sending the signal to small robots through a wireless link.

Already, the robots are able to walk in response to brain-organoid signalling. The team's next goal is to enable the brain organoids to receive input from the robots, which they aim to accomplish by the end of 2019. Muotri hopes the brain organoids will then be able to learn and adapt to their environment, "allowing us to test the impact of the Neanderthal variants on a physiologically relevant network". He thinks that his approach will eventually help to reveal crucial steps in the evolution of the modern-human brain, as well as some of the trade-offs required. "This strategy could not only illuminate how specific DNA alterations led to a highly sophisticated human social mind, but also shed light on the origins and causes of mental illness," he says.

From model brains to population-wide genomic analyses, researchers are taking advantage of clues from Neanderthals and their ancient crosses with anatomically modern humans. "To shed new light on the biological mechanisms underlying recent evolutionary changes to early brain development in our own lineage," Gunz says, "we turn to our closest extinct relatives, the Neanderthals." ■

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1. Cugola, F. R. *et al. Nature* **534**, 267–271 (2016).
2. Green, R. E. *et al. Science* **328**, 710–722 (2010).
3. Gunz, P. *et al. Curr. Biol.* **29**, 120–127 (2019).
4. Ponce de León, M. S. *et al. Curr. Biol.* **26**, R665–R666 (2016).
5. Gregory, M. D. *et al. Sci. Rep.* **7**, 6308 (2017).