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Feature

Intelligent life without bones

The first genome sequence of a cephalopod species, together with comprehensive functional annotation, offers a glimpse at how nature achieved complex functions and indeed intelligence in a lineage independent of vertebrates. It thus allows a more general view on complex life that could be extrapolated beyond the confines of life on Earth. **Michael Gross** reports.

From the Hydra of Ancient Greek legend to the Kraken of Norse myths, many-armed sea monsters have loomed large in the imagination of many sea-faring cultures. Through to the 19th and 20th century, writers from Victor Hugo and Jules Verne to H.G. Wells echoed these archaic fears and helped to keep the bad reputation of cephalopods alive.

The imagined horrors of those descriptions were mainly based on dead animals washed up on the shores or on rare encounters with fishermen. Only in the second half of the 20th century did underwater observation and filming by pioneers like Jacques Cousteau (1910–1997) begin to reveal cephalopods as a fascinating and complex group of animals.

Observations of intelligent behaviour of animals held in captivity — not least their uncanny ability to escape from aquariums — added to the re-evaluation, but to this day their scientific appreciation lags behind that of vertebrates, as witnessed by the lack of official conservation status and the late entry to the genome club.

Functional genomics now offers additional ways to study in detail a whole range of advanced functions that evolved in cephalopods independently of the comparable abilities in vertebrates, including the sophisticated camera eye, the active and movable camouflage patterns, and not least, their flexible intelligence.

Evolution

The sequencing of the genome of the California two-spot octopus, *Octopus bimaculoides*, was initiated by Nobel laureate Sydney Brenner, the founding president of the Okinawa Institute of Science and Technology (OIST) at Okinawa, Japan (Nature (2015) *524*, 220–224). It represents the first genome from the class Cephalopoda, which comprises nautiluses and ten-armed squids as well as octopuses.

The invertebrates the authors used as reference points for comparative genomics are the limpet (conical snail) *Lottia gigantea*, the polychaete annelid worm *Capitella telata*, and the cephalochordate (lancelet) *Branchiostoma floridae*.

The OIST researchers estimate the division between octopuses and squids to date back to 270 million years ago, broadly similar to previous estimates reviewed by Björn Kröger and colleagues (Bioessays (2011) *33*, 602–613). Nautiloids, of which only two genera with six species are known to have survived, separated from the coeloids (octopuses, cuttlefish and squids) some 416 million years ago. In geological timescales, that was just before our own early vertebrate ancestors started to conquer dry land and grow limbs, some 380 million years ago (Curr. Biol. (2013) *23*, R419–R421). Nautiloids kept their distinctive spiral shell, which has barely changed in the fossil record over hundreds of millions of years, as a buoyancy device, while the surviving coeloid lineages reduced and internalised theirs and in many cases lost it entirely. (Ammonites, which are closely related to coeloids and widely known as fossils, kept theirs but disappeared in the mass extinction that also claimed the dinosaurs.) The emergence of cephalopods as a lineage distinct from other types of molluscs is believed to have occurred around 530 million years ago, with the soft-bodied organisms emerging from their shells.

As bony fishes and later on terrestrial vertebrates evolved complex functions based on the support of more complex skeletons, it is ironic that cephalopods gained complexity by liberating themselves from their ancestral mollusc shells. In competition with early fishes, they emerged as fast-moving, flexible predators with keen eyes and sharp wits. The functional analysis of the genome now enables researchers to elucidate how these abilities could evolve outside the vertebrate subphylum, where we would be more likely to expect them.

Eye and brain

In a remarkable example of convergent evolution, cephalopods have independently evolved camera

Photogenic predator: Coeloid cephalopods, a group of shell-less molluscs, evolved at around the time when vertebrates started to conquer land. As visual predators, they competed against early fishes and developed remarkable complexity of body and behaviour. The photo shows the octopus *Wunderpus photogenicus*, found in shallow waters from Indonesia to the Philippines. (Photo: Roy L. Caldwell.)

Brood care: Female octopuses like this *Wunderpus photogenicus* look after their spawn but stop feeding and typically die soon after the offspring hatch. (Photo: Roy L. Caldwell.)

eyes — including a lens, a vitreous body, and a retina — as sophisticated as those found in mammals like ourselves. In some respects, they are even superior. For instance, a fundamental design flaw of our eyes is that, for reasons related to the evolutionary precursors of the vertebrate eye, the neural connections pass in front of the retina, leaving a blind spot. In the cephalopod eye, in contrast, the wiring is behind the receptor field, just as an intelligent engineer would design it.

The cephalopod brain, which takes up a comparable fraction of body mass as those of birds and mammals (and much more than reptiles and fishes), is also wired a bit differently from what we are used to. It also sports a remarkable donut shape with the oesophagus passing through the central hole — a situation that can cause problems if an octopus swallows bony prey that proves too large to pass through the brain.

More importantly, the nervous system appears to be more decentralised with more of its neurons located in the periphery. This corresponds to the relative autonomy of the arms, which appear to operate on general guidelines from central command but make their own decisions in the details (Curr. Biol. (2015) *25*, 1195–1200). Thus, the octopus's head doesn't necessarily know what its arms are up to, and can only check up on them visually. It has been argued that, due to the remarkable behaviour repertoire of the arms, keeping track of all of them would be too complex a task for the size of the (already large) central brain. As the group of Binyamin Hochner at the Hebrew University of Jerusalem, Israel, has shown, their suction cups are equipped with chemisensors that can detect the proximity of the animal's own skin, which help to ensure that the arms don't bump into each other (Curr. Biol. (2014) *24*, 1271–1275).

The genome analysis has started to reveal some clues to the neural complexity of cephalopods. While most gene families involved in neurotransmission appear to be fairly typical of molluscs and related invertebrates, and not all that different from vertebrates, the OIST researchers found a few characteristic expansions, such as the family of sialic acid vesicular transporters (sialins), the protocadherins, and the C2H2 zinc finger proteins, all of which are linked to information processing.

The protocadherin gene family, for instance, appears to have expanded significantly even after the divergence from the squid lineage. Transcriptome analysis shows that this gene family plays an important role in brain organisation in both coeloid cephalopod lineages as well as in vertebrates, another example of convergent evolution.

The convergence seen in details like protocadherins and eyes, however, doesn't offer explanations of how the complex brains and bodies of cephalopods and vertebrates arose in the first place. Before this genome study, several researchers had hypothesised that the evolution of both were made possible by whole-genome duplication. However, while the evidence for such a duplication in early vertebrate evolution is strong, the genome analysis showed no trace of such an event in the cephalopods. While it still cannot be ruled out, the parsimonious theory is that it didn't happen. Instead the study found a remarkable number of mobile genetic elements (transposons) in the octopus genome, making up nearly half of it. Many of them were found to be active in neural tissues, where they may have an as yet undetermined role in learning and memory.

Intelligence and behaviour

Octopuses display a wide range of behavioural responses that have long fascinated researchers and laypeople alike. The octopuses' first line of defence, for instance, is their remarkable ability at camouflage. Using muscles that can alter the size of pigmented cells in their skins, they can mimic a wide range of substrates and even produce moving patterns such as the famous 'passing cloud' phenomenon. Those living on coral reefs can also adapt their skin texture to resemble a rough background. Others may pretend to be plants or walk away on two legs to fool any predators specifically looking for eight-legged prey. Should these approaches fail, there is always the powerful jet propulsion mechanism to speed them away, while leaving behind a cloud of black 'ink' (coloured by melanin, the pigment in our skins), which blinds the attacker and may trick them into attacking the ink cloud instead of the absconding cephalopod.

A recent paper has added evidence of further intriguing behaviours displayed by larger Pacific striped octopus in captivity. Roy Caldwell from the University of California at Berkeley and colleagues have studied and filmed 24 individuals (PLoS One (2015) *10*, e0134152; six videos are available in the supplementary materials of the paper: http://bit.ly/1UdhRgb). Among the surprising behaviours they observed was a mischievous hunting strategy that involved the octopus extending a single arm over and beyond the targeted crustacean and nudging it from the far side, thereby startling it to flee right towards the other arms.

The researchers were also surprised to find mating pairs willing to share a den and engaging in beak-to-beak close contact, unlike the more widely observed 'arm's-length' process whereby the male extends a specialised arm to transfer his sperm to the female's mantle cavity. The researchers also observed one example of inking during mating. While octopuses would typically spawn once and then starve to death, the researchers also observed extended spawning in this population. One of the co-authors, Arcadio Rodaniche, had already observed and described some of these behaviours in this species in Panama in the 1970s, but had his manuscript rejected because the observations were so unexpected and at that time not backed up by video evidence.

A recent study of mating behaviour in vampire squid (*Vampyrotheutis infernalis*) has demonstrated multiple reproductive cycles for the first time in a coeloid cephalopod (Curr. Biol. (2015) *25*, R322–R323), highlighting that much of the behavioural repertoire of cephalopods remains to be explored. Of the more than 300 extant octopus species, many have never been observed in the wild, so any generalisations are bound to be at danger from new discoveries.

Octopuses have always been prone to surprise their human observers. Early filming by Jacques Cousteau's team showed how they could uncork a glass bottle containing a shrimp. They have also opened screw-cap containers and memorised different escape routes from similar mazes. Octopuses have been filmed carrying away coconut shells to use as dens and carrying tentacles of the Portuguese Man o' War (*Physalia physalis*) for their defence. And many a collector has put an octopus into a sealed container, only to find it empty a few hours later.

Mischief maker: Among the behaviours recently observed in octopuses is a 'tap on the shoulder' with one arm approaching from the far side, startling the prey and chasing it into the other seven arms. (Photo: Roy L. Caldwell.)

As the most recent common ancestor of vertebrates and cephalopods would have been a worm-like microscopic organism with no brain worth mentioning, the remarkable intelligence of these tentacle bearers must have evolved convergently with vertebrate intelligence. It offers a useful external reference for investigations into the nature of intelligence and the ecological driving forces that may lead to its development.

In a recent review of cephalopod cognition in its evolutionary context, Joseph Vitti from Harvard University, USA, concluded that "the most critical factor in selection for domain-general intelligence is variability and hostility in the environment". Mammals, birds and cephalopods have all responded to the variable challenges they faced by developing flexible cognitive processes that we recognise as intelligence (Biosemiotics (2013) *6*, 393–401). The availability of cognitive hardware, such as the sophisticated visual apparatus, only plays a secondary role, according to Vitti.

Alien life?

Cephalopods are sometimes described as 'aliens' in the media (and even in the OIST press release heralding the genome paper), which mainly reflects the fact that we have very little experience with them and that we don't expect to see complex functions and intelligent behaviour in a mere mollusc. Their sheer exoticism also explains their prevalence in horror movies and science fiction.

On a more serious note, however, they do offer us a glimpse of an alternative form of intelligent life outside the vertebrate norm that we are used to. As Sydney Brenner said on release of the genome paper, "they were the first intelligent beings on the planet". We can't be quite sure, but before birds and mammals rose to prominence, the most intelligent life form on planet Earth may well have been a cephalopod, and that situation may have lasted for more than a hundred million years.

They also broaden our horizon concerning the possible trajectories that the evolution of life might have taken on Earth or other planets. If the evolution of bony skeletons had failed to take off — due to a shortage of the requisite minerals, say — the comforting message is that there is intelligent life without bones. And if we ever discover complex life forms on other planets, they may well come with soft bodies and more than four limbs.

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