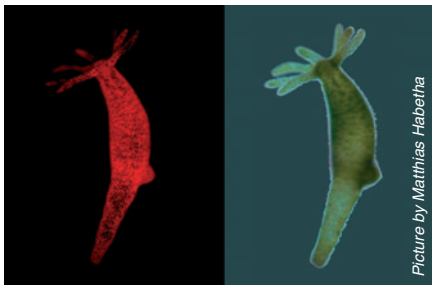
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

HYDRA FINDS A PLANT GENE IN THE FAMILY TREE



The tiny green tentacles of *Hydra* polyps are a familiar sight for most zoology students. Matthias Habetha and Thomas Bosch at Kiel University now have something to add to the textbooks: while scrutinizing the genes that underlie *Hydra*'s relationship with an algal symbiont, they discovered that *Hydra viridis* expresses a plant gene (p. 2157)!

Habetha and Bosch are intrigued by the genetic basis of the relationship between *Hydra viridis* and *Chlorella*, the algal symbiont that lives in *Hydra*'s epithelial cells and gives the creature its familiar green colour. Since all eukaryotic cells are products of symbiosis between once free-living bacteria, 'studying this ancient inter-kingdom communication process can provide us with interesting evolutionary insights,' Bosch says. Habetha and Bosch want to know which genes are activated when *Hydra* harbours its symbiont, to find out what regulates this intimate coexistence.

To screen for symbiosis-specific genes, Habetha and Bosch examined differences in gene expression between symbiotic *Hydra* and aposymbiotic *Hydra* – those that had been artificially induced to give up their symbionts. They identified six genes that are only expressed in symbiotic *Hydra*, and concluded that these genes are specific to symbiosis. But they were in for a surprise; when they searched animal genomes for homologues of the six genes, they found that one of the newly discovered genes bears no resemblance to any animal genes. Instead, it is closely related to plant peroxidase genes; they had found a plant gene in an animal genome! 'We were so surprised that at first we thought that our samples had been contaminated with *Chlorella* DNA,' Bosch recalls. But when they checked *Chlorella*'s genome, they couldn't find the gene – it really was in the *Hydra* genome.

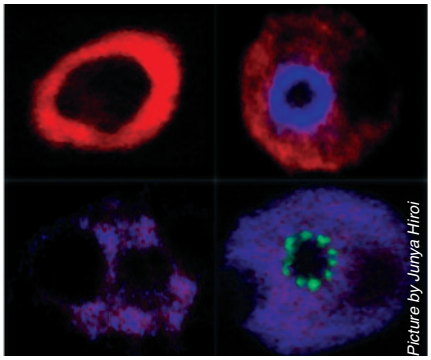
So how did it get there? Habetha and Bosch suggest that, at some point in evolution, a symbiont transferred some of its genetic material into *Hydra*'s genome. But they knew that *Chlorella* couldn't be the donor, because they hadn't found the peroxidase gene in its genome. 'So the gene probably came from an earlier plant symbiont, which was later replaced by *Chlorella*,' Bosch concludes. They had another clue: *Hydra*'s peroxidase gene lacks introns (segments of DNA that don't code for proteins) but all the modern plant homologues of this gene have introns. Since introns are spliced out when DNA is transcribed into mRNA, 'the lack of introns suggests that the gene transfer occurred *via* mRNA,' Bosch explains.

Habetha and Bosch also discovered that the peroxidase gene is only expressed in *Hydra* undergoing oogenesis. Bosch isn't surprised that a symbiont-related gene is expressed at this point in the life cycle. '*Chlorella* is transmitted by oogenesis. The algae ensure that they end up in new polyps by crawling into developing oocytes,' Bosch says. He speculates that horizontally transferred genes like the one just identified 'might be involved in oocyte formation to ensure the survival of the symbiont.' Bosch expects to find more horizontally transferred genes once *Hydra*'s genome sequencing project, which is underway in the US, is completed.

10.1242/jeb.01671

Habetha, M. and Bosch, T. C. G. (2005). Symbiotic *Hydra* express a plant-like peroxidase gene during oogenesis. *J. Exp. Biol.* **208**, 2157-2164.

SALINITY WORKOUT FOR ION-PUMPING CELLS



Fish face some serious osmotic challenges; freshwater fish soak up water while those in the sea lose water to the salty surroundings. Luckily, fish can be flexible in their uptake or excretion of ions; some species switch their gills' ion pumps when they move between fresh and salty water. But how do embryos that don't yet have working gills cope with salinity changes?

Junya Hiroi and his colleagues already knew that the yolk-sacs of tilapia embryos contain ion-pumping cells. Now, they have discovered that tilapia embryos deal with salinity challenges by changing the ratio of different ion-pumping cell types in their yolk-sacs (p. 2023).

Hiroi explains that three main ion transport proteins regulate a fish's internal salt levels. To see where these transporters are located in tilapia yolk-sac cells, he used antibodies labelled with three fluorescent colours, each of which binds to a specific ion transporter. Each antibody's fluorescent colour shows up wherever its specific ion transporter is present in a cell. 'This was the first time that we could see all three transport proteins at the same time in one cell,' Hiroi says.

Watching the appearance or disappearance of these fluorescent stains over time would allow Hiroi to see how the number of yolk-sac ion-transporting cells changed as the embryos coped with salinity stress. Hiroi first incubated some tilapia eggs in freshwater and others in seawater. After a few days he moved the freshwater embryos to seawater, and *vice versa*. To see how the embryos' yolk-sac cells changed over time, he incubated the yolk-sacs with the three fluorescent antibodies and examined the stained yolk-sacs under a microscope before he transferred the embryos, and repeated this process 1, 2 and 3 days after transfer.

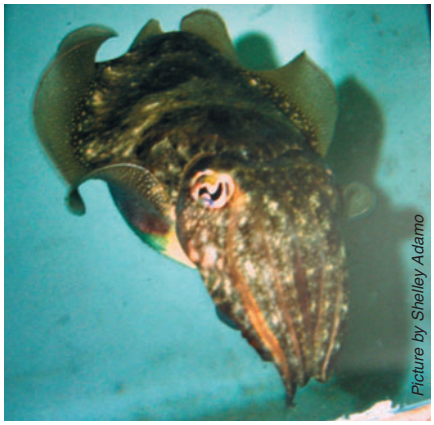
Hiroi was able to classify four different cell types based on the combinations of ion transporters that he could see in the cells. In the freshwater embryos he saw three cell types, which he labelled type-I, type-II and type-III. When Hiroi moved embryos from fresh to seawater, he saw that the number of type-III cells decreased while type-IV cells appeared and grew in number. This suggests that type-IV cells are ion-secreting cells that help the embryos survive in salty water by pumping out salts. In contrast, seawater embryos started out with many large type-IV cells and only a few type-I and type-III cells. When Hiroi moved seawater embryos to freshwater, he saw that type-IV cells disappeared, type-III cells became more numerous, and type-II cells appeared and also multiplied. Hiroi concludes that type-II cells are freshwater-specific ion absorbers, which scavenge ions to replace lost salts when embryos are in freshwater.

The patterns of mysteriously appearing and disappearing type-III cells suggest an exciting possibility; type-III cells might transform into type-IV cells when freshwater embryos suddenly find themselves in seawater. 'But we can't say that this is definitely happening,' Hiroi admits. He hopes to take a closer look at the function of type-III cells once antibodies are developed that can distinguish between absorptive and secretory isoforms of one of the ion transporters found in type-III cells.

10.1242/jeb.01673

Hiroi, J., McCormick, S. D., Ohtani-Kaneko, R. and Kaneko, T. (2005). Functional classification of mitochondrion-rich cells in euryhaline Mozambique tilapia (*Oreochromis mossambicus*) embryos, by means of triple immunofluorescence staining for Na^+/K^+-ATPase, $Na^+/K^+/2Cl^-$ cotransporter and CFTR anion channel. *J. Exp. Biol.* **208**, 2023-2036.

PEERING INTO CUTTLEFISH



It's been known for some time that cuttlefish have contractile veins, but only from dissections; nobody had ever seen them contract in a live, free-swimming cuttlefish. To investigate this remarkable feature, Alison King and her colleagues at Dalhousie University and the Scripps Institution of Oceanography developed an ingenious set-up to see cuttlefish circulatory systems in action (p. 2071).

King wanted to know how blood returns to the cuttlefish heart. Most of our veins are conveniently located in muscles, and their contractions squeeze blood back to our heart. But the large cuttlefish veins aren't surrounded by muscle. Instead, they sit in a body cavity enclosed by the mantle - a big, muscular body wall that ventilates the cuttlefish's gills. It's generally assumed that the large veins are compressed by increased pressure in the cavity caused by the mantle's contractions, and that this pushes blood back to the cuttlefish's heart. But is this really what happens? Radiologist Matthias Schmidt astutely suggested that King could try using ultrasound to find out.

King and Schmidt began experimenting, but soon hit a snag; they found that the cuttlebone in cuttlefish backs is opaque to ultrasound. Realising that she'd have to go underneath the cuttlefish to see anything, King designed a plastic cylinder propped up with struts. To get ultrasound images of the insides of the cuttlefish happily resting in the cylinder, King pressed an ultrasound transducer against the bottom of the cylinder and hoped the creature would sit still long enough for her to get 30-second video clips. It worked; King could finally take a peek inside cuttlefish. But she had to make sense of the seething mass of cuttlefish insides. Poring over an atlas of cuttlefish body parts, she struggled to decipher her upside-down moving images. It was worth it. 'For the first time, we could see blood vessels changing shape in live cuttlefish,' King says. 'It was magical seeing physiology in action.'

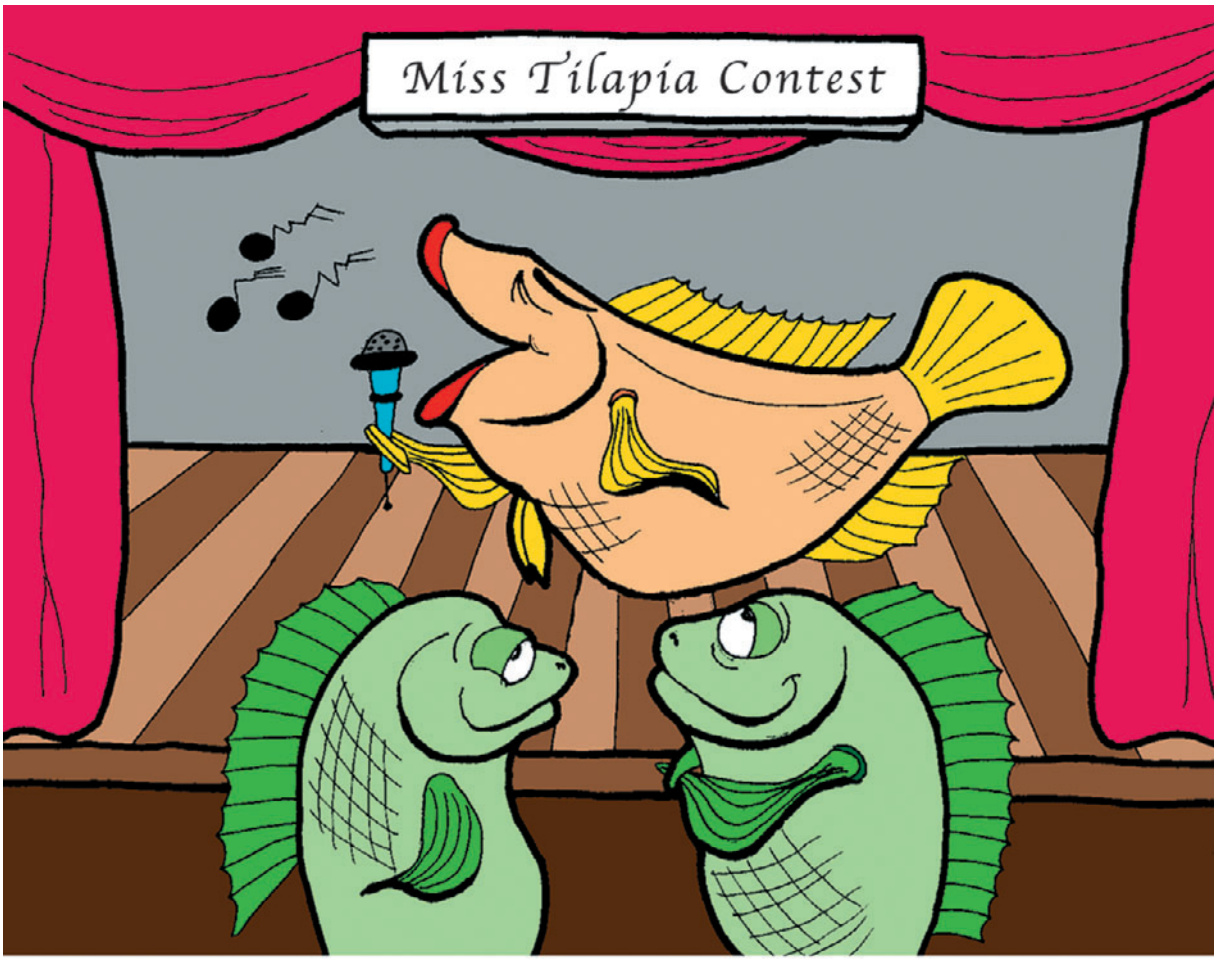
So, does increasing mantle cavity pressure compress the veins, pushing blood to the heart? If it does, the contractions of the anterior and lateral venae cavae (two of the major veins that deliver oxygen-depleted blood to the heart) should be in sync with the mantle's contractions. But King saw that the lateral venae cavae and the mantle contract at different rates. Since they are out of sync, the mantle can't be compressing the lateral venae cavae. Taking a closer look at her real-time images, King saw peristaltic waves moving along the anterior and lateral venae cavae; the veins contract on their own! So it's unlikely that pressures created by the mantle compress the veins, because then 'we'd expect the veins to collapse as a unit, rather than progressively along their length,' King says. She suggests that 'actively contracting veins aid the return of blood to the cuttlefish heart.'

But King also noticed that the anterior and lateral venae cavae contract at different rates, which could spell disaster. The two veins are connected, so if one relaxes and expands while the other contracts, blood could flow in the wrong direction. Cuttlefish solve this potential problem in the same way we do; King discovered a new valve (which she has dubbed the Wells valve) sitting between the venae cavae, which ensures that blood always flows towards the heart.

10.1242/jeb.01670

King, A. J., Henderson, S. M., Schmidt, M. H., Cole, A. G. and Adamo, S. A. (2005). Using ultrasound to understand vascular and mantle contributions to venous return in the cephalopod *Sepia officinalis* L. *J. Exp. Biol.* **208**, 2071-2082.

SMELLS FISHY…



Frank Fish is a professor at West Chester University

Although her talent left much to be desired, to the male judges she had that sweet smell of success

A male tilapia's idea of a great chat-up line is to lose control of his bladder. Whether the object of his affections finds this chemical courtship appealing is questionable. But how does a male decide which lady to woo? Peter Hubbard and his colleagues at the Universidade do Algarve in Portugal suspected that male tilapia sniff out which females are about to spawn (p. 2037).

The group decided to test whether males can tell the difference between scents from pre-ovulatory females (those about to spawn) and post-ovulatory females (those that have already spawned). To see if body fluids from different females titillated males' olfactory receptors, they trickled water containing urine and faeces from pre- and post-ovulatory females past males' olfactory epithelia. Watching electro-olfactograms produced from the recordings of electrodes placed inside males' nostrils, they saw that males clearly find a whiff of pre-ovulatory female much more exciting than that of a post-ovulatory fish. The group suggests that pre-ovulatory females release some indicator of their sex appeal in their body fluids, while post-ovulatory females don't.

But do male tilapia also court pre-ovulatory females more enthusiastically? To find out, the group placed females in the males' tanks and kept a close eye on the males' behaviour. Sure enough, pre-ovulatory females found themselves fending off ardently urinating suitors, while post-ovulatory females didn't entice males to empty their bladders. So, female tilapia release sex pheromones to announce to their admirers that they're ready to spawn, and males passionately respond with their own chemical broadcast.

10.1242/jeb.01672

Miranda, A., Almeida, O. G., Hubbard, P. C., Barata, E. N. and Canário, A. V. M. (2005). Olfactory discrimination of female reproductive status by male tilapia (*Oreochromis mossambicus*). *J. Exp. Biol.* **208**, 2037-2043.

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