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

WHAT MAKES DEVILS HOLE PUPFISH SPECIAL?

Picture by Sean Lema

Deserts aren't the most obvious aquatic environments, but rare water holes and fissures crop up in most deserts; even Death Valley in the USA. Even more remarkably, some of these waterholes have been colonised by fish, and one, the 3 m×7 m Devil's Hole, is home to the planet's entire population of *Cyprinodon diabolis*, better known as the Devils Hole pupfish. Sadly the hole's vulnerable residents became a 'cause-célèbre' in the late 1960s, when water-pumping activity threatening the pupfish's survival. Activists mobilised, and a 1976 landmark US Supreme Court ruling forced the pumping to stop. But by then the Devils Hole pupfish was in serious danger, so conservationists set up 3 refuges to establish backup populations. However, within 5 years, ecologists noticed that the refuge populations were losing some of the Devil's Hole distinctive characteristics; the pupfish's heads and eyes were smaller than those of the original population and their bodies became deeper. Curious to know whether environmental factors could be driving the fish's morphological modifications, Gabrielle Nevitt and Sean Lema decided to see if diet restriction could alter morphological characteristics in a close, but unthreatened, relative; the Amargosa River pupfish (p. 3499).

Hiking to the river's Tecopa Canyon, Lema collected Amargosa River pupfish before returning to Nevitt's lab in the University of California at Davis. Collecting freshly laid eggs, Lema waited for the larvae to hatch before dividing them into three groups; one was fed an unrestricted diet, another a moderate diet and the third on a severely restricted diet. Having set each of the tanks to the Devil's Hole temperature, 33°C, Lema noticed that the tanks' temperatures varied a little, so he factored temperature into his analysis of the fish's development too. Monitoring the fish over 126 days, Lema recorded the size of their heads and body depth relative to their body length. Sure enough, fish on the restricted diet had restricted growth and relatively large heads and shallow bodies, just like the Devils Hole pupfish. But most surprisingly, the fish that had been exposed to water that was just 1°C warmer than the experimental temperature failed to develop pelvic fins, just like the true Devils Hole pupfish. Both temperature and diet restriction had a big effect on the developing fish's morphology. 'We hadn't expected such a strong impact on fin development' admits Lema.

So what could be driving these drastic physical changes? Lema explains that Don Brown's 1997 *Proc. Nat. Acad. Sci.* publication suggested that low thyroid hormone levels could prevent zebrafish larvae from developing pelvic fins. Were the underfed pupfish's thyroid hormone levels low? Measuring the fish's whole body hormone levels, Lema found that they were considerably lower than the well-fed fish.

Next Lema and Nevitt decided to see what happened to developing fish larvae when their thyroid levels had been lowered pharmacologically, and were amazed to see that the fish's growth on a good diet was unrestricted, but that they failed to develop pelvic fins. The team suspect that the Devils Hole pupfish's metabolism is significantly affected by their poor diet in hot water, naturally reducing their thyroid hormone levels, resulting in their unique appearance.

But what hope does this offer for the Devils Hole pupfish's survival? Lema suspects that the fish's best hope is a carefully controlled captive breeding program, and with their current numbers at record low levels, the fish have never before needed help more than they do now.

10.1242/jeb.02509

Lema, S. C. and Nevitt, G. A. (2006). Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. *J. Exp. Biol.* **209**, 3499-3509.

MOSQUITO SODIUM/PROTON EXCHANGER IDENTIFIED

A buzzing mosquito is an all too familiar, and unwelcome, summer sound; a hungry female is searching for her next blood meal. But once she has gorged herself, she faces two major challenges: dealing with the meal's enormous volume and high salt content. Sarjeet Gill explains that although many details of the membrane transport processes involved in processing the meal have been understood for several decades, the molecular identities of the transporters have remained elusive. Knowing that sodium transport across membranes in mammals is driven by sodium/proton exchangers, Gill and his colleagues at the

University of California, Riverside, decided to identify and clone the transporter (p. 3529) from the infamous mosquito: *Aedes aegypti*.

Basing his search on known sodium/proton exchangers from other organisms, Gill identified two key regions in these proteins that would help him to identify and locate the mosquito form of the gene. By successfully matching mRNA isolated from the insect's midgut and Malpighian tubules with these key regions, the team were able to isolate the elusive gene and clone it.

Next, the team went on to test the transporter protein's function. Cloning the new gene into sodium sensitive yeast cells that had lost sodium tolerance and all four of their own sodium transporters, the team found that the cell's sodium tolerance was restored by the mosquito gene, but only to an extent. It seemed that the mosquito transporter compensated only for the loss of the yeast's plasma membrane sodium transporter, but didn't compensate for the lost vacuolar sodium transporter.

Knowing that the transporter probably exchanges protons for sodium ions, the team decided to see if the protein restored acidity tolerance to a mammalian cell line that had lost its own sodium/proton exchanger. Cloning the new gene into the cells and exposing them to an acidic environment, the team found that the mosquito gene restored the cell's pH tolerance. Finally the team tested the mammalian cell's ability to take up radioactive sodium, and found that only the cells that had received the mosquito gene incorporated the hot sodium.

Having convinced themselves that the protein expressed by the new mosquito gene had all the characteristics of a sodium/proton exchanger, the team decided to identify locations in the insect's body where the gene is expressed. Designing an antibody that recognised a portion of the enormous transporter protein, the team probed major tissues involved in processing both fluid and ions, including the gut and Malpighian tubule, and found that the transporter protein was localised in the basal plasma membrane of the Malpighian tubule, the midgut, and regions of the gastric caeca. The team admit that they were surprised by the protein's occurrence in the Malpighian tubule's basal membrane on two counts; it is usually found in apical membranes in vertebrate kidneys, and all Malpighian tubule ion transport models developed to date have placed cation/proton exchangers in the apical membrane. The team want to follow up this discovery by finding out how the transporter is trafficked within the insect's Malpighian tubules, and to refine ion transport models, which have previously overlooked the occurrence of sodium/proton exchangers in the basal membrane.

10.1242/jeb.02508

Pullikuth, A. K., Aimanova, K., Kang'ethe, W., Sanders, H. R. and Gill, S. S. (2006). Molecular characterization of sodium/proton exchanger 3 (NHE3) from the yellow fever vector, *Aedes aegypti*. *J. Exp. Biol.* **209**, 3529-3544.

SYMBIONT'S BODY BUFFERS

Until the late 1970s, scientists were convinced that all life on earth depended largely on the sun for energy. However, when geologists stumbled on the first deep-sea hydrothermal vents, at depths well beyond light penetration, they discovered thriving ecosystems powered not by sunlight, but chemosynthesis. Peter Girguis explains that symbiotic organisms living by the hydrothermal vents derive energy by chemosynthesis of toxic sulphide compounds released from the vents, to power the conversion of carbon dioxide into sugars for consumption by their tubeworm hosts. The environment inhabited by these symbiotic creatures is also incredibly dynamic, with mixing between the icy ocean waters and scalding deep-sea geysers causing sulphide levels to vary from almost zero to several millimolar within a matter of moments. Which made Girguis and James Childress wonder how symbiotic *Riftia pachyptila* tubeworms sustain some of the highest growth rates ever measured when their energy supply is so variable. Girguis and Childress decided to find out how varying the tubeworm's environment affected the symbiont's metabolism (p. 3516), but first they had to build an oceangoing high pressure respirometer to keep the tubeworms happy onboard ship while they made their sensitive measurements.

Childress and Girguis attached a mass-spectrometer to a high-pressure aquarium, but delivering high-pressure seawater to the high-vacuum mass-spectrometer posed enormous technical challenges. Having successfully plumbed the two together, the team heading out into the Pacific Ocean to collect tubeworms and begin measuring their sulphide, carbon and oxygen uptake rates over a range of physiological conditions.

Recreating the tubeworm's hydrothermal vent environment by bubbling carbon dioxide, hydrogen sulphide and oxygen through seawater before delivering it to the high-pressure aquarium, they supplied the symbionts with oxygen over a range of 40 mmol l^{-1} to 210 mmol l^{-1} at fixed sulphide and carbon levels and found that the animal's oxygen consumption rate rose continually as the oxygen supply increased. The team also increased the animal's carbon dioxide supply up to a maximum of 8 mmol l^{-1}, while keeping their sulphide and oxygen levels constant, and found that the animal's carbon consumption rates also increased up to a maximum level. But most importantly, how did the animals respond to fluctuations in their sulphide supply?

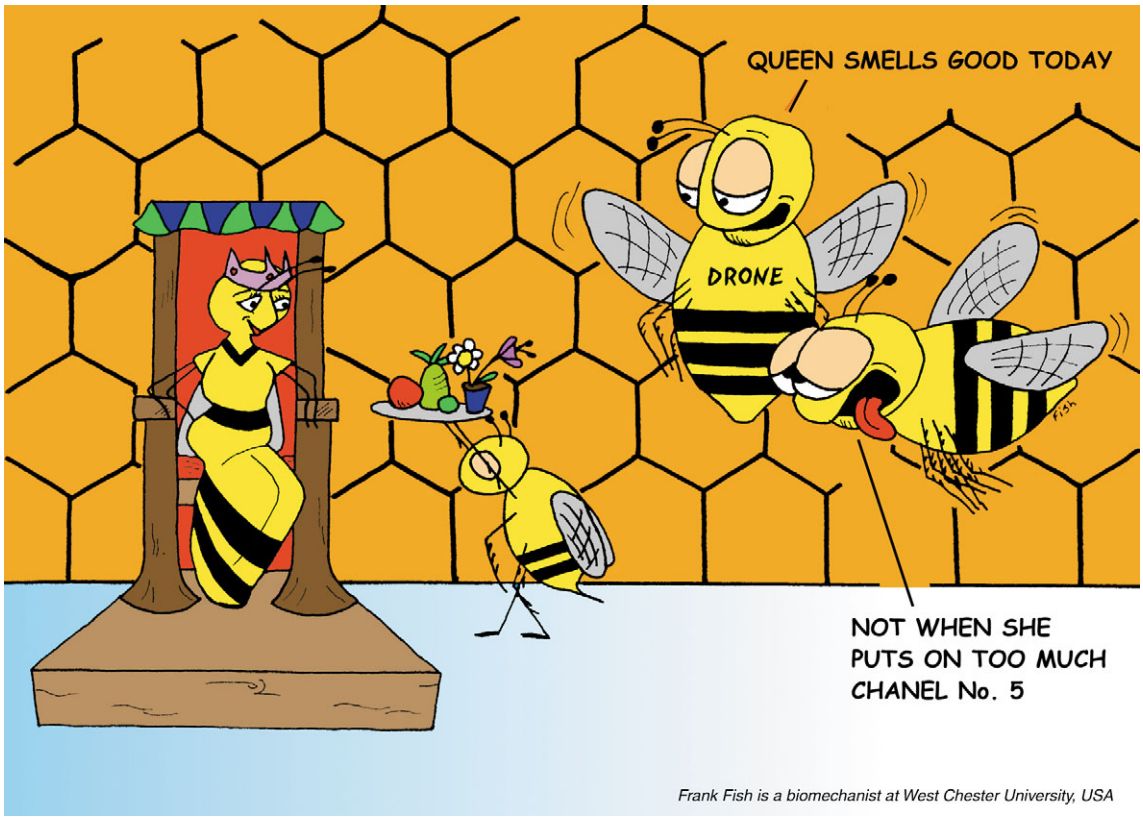
Knowing that sulphides come in two forms (toxic H_2S and less toxic HS^-), Girguis and Childress measured the tubeworm's sulphide uptake rates by switching between the two forms at pH 5.66 and 7.48 and found that surprisingly the tubeworms were able to take up both forms. They suspect that the toxic H_2S form is converted to the safer HS form once in the animal's blood. They also found that the tubeworm's uptake rate increased as the sulphide and oxygen supplies increased, but dropped dramatically once environmental sulphide levels became dangerously high at 700 mmol l^{-1}.

The most startling results came when the team dropped the tubeworm's sulphide supply to zero, expecting to see carbon uptake drop too. However the symbionts continued to take up carbon dioxide and synthesise sugars, even though they had lost their power source. The team suspects that the tubeworm had stored enough sulphide in its body to keep its symbiotic lodger well supplied when sulphide in the environment became scarce. Which probably explains why *Riftia* thrives its turbulent surroundings, using its body as a buffer to protect its bacterial lodger from environmental fluctuations.

10.1242/jeb.02510

Girguis, P. R. and Childress, J. J. (2006). Metabolite uptake, stoichiometry and chemoautotrophic function of the hydrothermal vent tubeworm *Riftia pachyptila*: responses to environmental variations in substrate concentrations and temperature. *J. Exp. Biol.* **209**, 3516-3528.

MACROGLOMERULUS PICKS UP QUEEN'S SCENT



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It's no coincidence that hives are a byword for industry; female worker bees scurry around tending to their queen, yet male drones hardly seem to contribute at all, their existence is geared solely to mating with the queen. Jean-Christophe Sandoz, from the Université Paul Sabatier in Toulouse, France, explains that apart from their different social roles, the male drone's olfactory processing antennal lobe is quite different from the female's; four of the drone's odour-sensitive glomeruli are enlarged relative to the ordinary glomeruli, and it had been suggested, but never proved, that they were responsible for the male's sensitivity to the queen's pheromones.

Curious to know how the antennal lobe responds to everyday hive odours (p. 3587), Sandoz used calcium imaging to visualise the activity of glomeruli on the antennal lobe's surface in response to various scents. Testing the effects of several queen pheromone components, Sandoz found that main constituent, 9-ODA, triggered a response in the enlarged MG2 glomerulous only; MG2 responds specifically to the queen's pheromone. Sandoz suggests that 'most, if not all, of the olfactory sensory neurones tuned to [the queen pheromone component] 9-ODA project to this macroglomerulus'.

10.1242/jeb.02507

Sandoz, J.-C. (2006). Odour-evoked responses to queen pheromone components and to plant odours using optical imaging in the antennal lobe of the honey bee drone *Apis mellifera* L. *J. Exp. Biol.* **209** 3587-3598.

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