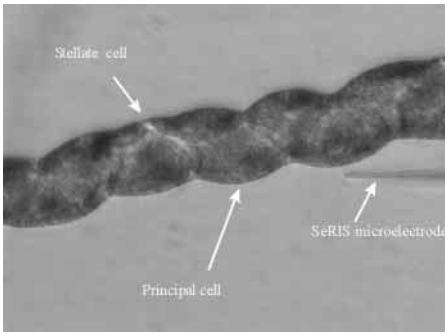
IN THIS ISSUE



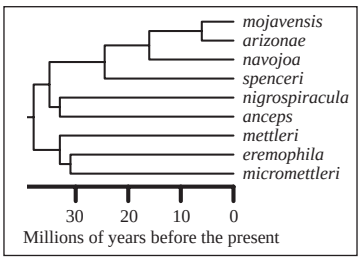
Kidneys and Currents (p. 2289)

Whatever your size, kidneys are the key to homeostatic regulation, continually pumping ions and water back and forth across the epithelium layer. *Drosophila*'s basic filtration unit is the Malpighian tubule, which is composed of two distinct cell types, stellate and principal cells. Principal cells are cation transporters that are found throughout Malpighian tubules. Stellate cells are localised to the main segment of the tubule, and had only been shown to transport chloride ions. Although visually indistinguishable, Julian Dow's team found that principal cells are genetically heterogeneous. This led Dow to wonder whether individual principal cells were fulfilling different functions, but to test this, someone would need to make incredibly accurate measurements of tiny ion fluxes from individual cells. Enter Mark Rheault and the mighty micro-electrode!

Rheault used self-referencing ion-selective (SeRIS) microelectrodes that had been developed by teams working with Peter Smith at Woods Hole and Joe Kunkel at Amherst. The approach measures the voltage at two positions close to the tubule wall. These potentials are then converted into concentrations and ion fluxes for specific cations. Using Alan Shipley's incredibly accurate positioning systems, Rheault was able to measure voltage drops as tiny as 10 µV over 100 µm with impressive accuracy. He compared stimulated and unstimulated tubules to see whether all principal cells responded equally, with the aim of characterising the ion transport properties of Malpighian tubules at cellular resolution.

It had always been unclear whether or not the tubule's distal end was involved in K^+ transport. Using the SeRIS system, Rheault was able to establish that this is the only region of the Malpighian tubule that doesn't transport potassium ions. When he measured the transport properties of principal cells at different sites along the tubule, he saw that even though they seemed identical, they did not transport potassium equally. Despite their apparent homogeneity, principal cells are functionally heterogeneous. This proves the method to be an incredibly powerful tool for probing epithelial ion transport. Rheault has measured ion fluxes that were completely impossible to detect by more traditional methods. The next question is what are the molecular differences that allow neighbouring cells to respond so differently?

The number of labs equipped to apply the method has proliferated in the last 4 years from a tiny handful to over 40, showing that although it can be fiddly to use, the returns more than compensate the effort invested.



Watering the Family Tree (p. 2331)

Comparative techniques are fantastically powerful when learning lessons in adaptation, but they only work if you compare like with like. 'The main limitation is that we've been comparing apples and oranges', says Allen Gibbs. He explains that comparisons between different organisms that live in different environments don't work because it's impossible to separate out the general differences between unrelated species and genuine metabolic differences that are needed for survival.

What if you wanted to find out how creatures survive arid climates? You'd try to find related species that occupy different habitats. In theory the only differences between them should be the adaptations that they need to survive a particular climate. Which is where *Drosophila* step in, because they have set up home in almost every environment on the planet and they have a well established family tree. Although it's not entirely clear what drove certain members of the fruit fly family to take up residence in the desert, they are obviously well suited to the desiccated climate. Comparing them with their temperately adapted cousins makes them the perfect family unit for answering the key questions of drought resistance. How do the desert flies tough the drought out, and did they move to the desert because they could or did they adapt after they arrived?

Gibbs and his colleague Luciano Matzkin teamed up to tackle the problem. They decided to mix phylogenetics with more traditional whole animal physiology to see if they could answer the 'how' and the 'when' of *Drosophila* water management. First, they collated all the available phylogenetic data to compile a complete family tree for 20 species in the study. Although the data came from a variety of sources, they all agreed incredibly well 'which gave us confidence' says Gibbs. Knowing how each branch of the family was related, they then tested how resistant each group was to desiccation, hoping to identify which of three alternative approaches to surviving dehydration had been adopted by the desert dwellers.

What Gibbs and Matzkin found was that desert flies survive the arid conditions because they've reduced their water loss rate. When you look at the family tree, their temperate cousins have serious water retention problems, so this is a strategy that the desert flies evolved in response to selective pressure. When Gibbs compared how tolerant the flies were to dehydration in general, he found that the desert flies could tolerate very low hydration levels, but not lower than closely related flies from a relatively humid environment. Looking at the way these flies are related showed that both groups diverged from ancestors that are probably drought resistant and passed the tolerance on to both family lines.

So the desert dwellers probably moved into an environment that they were already suited to, and once they'd arrived, they adapted further by conserving what little water they had so they could stick it out in the desert for generations to come. Now that the phylogenetics has proved that *Drosophila* desert survival tactics are a mixture of natural talent and learning, the next challenge is to identify how the desert flies have turned the tap off, and sealed the water in.

When the Going Gets Tough! (p. 2339)

It's a warm sunny day in the San Francisco Bay Area; the sailors are making the most of the onshore breeze and fishermen are waiting on the piers for the next bite. Meanwhile, life in the rest of the Bay isn't always quite so tranquil, at least not if you're a brine shrimp in the South Bay salt ponds.

Through its life cycle *Artemia franciscana* has to survive an incredible array of environmental stresses. No matter how salty it gets or whether their pond dries up completely, these little beasts somehow survive it all. And if you think mum and dad are tough wait 'til you see the kids! Brine shrimp embryos can develop along one of two pathways, depending on how rough it looks 'out there'. Approach one is used in clement

conditions, when they simply emerge fully-formed and free-swimming from their mothers. The second approach is reserved for the brine shrimp-equivalent of famine and pestilence. If the mother experiences physical stress, the embryos are programmed to switch everything off, shut down their metabolism, and go into a state of suspended animation (diapause). These little guys are so tough, you can send them into space, and they'll still hatch when returned to planet Earth.

The key event that precedes entry into diapause comes when the embryo begins to synthesise massive amounts of a protein called p26. This small protein is a chaperone that protects cellular proteins from degradation during stress. As the embryo enters diapause, 50% of the cell's entire supply of p26 relocates from the cytoplasm to the nuclei, but when the embryo reactivates and continues development, the chaperone exits the nuclei. If the embryo experiences a further bout of stress, the levels of nuclear p26 rise again. Surely p26 must be doing something fundamental in embryonic nuclei to be able to orchestrate such a catastrophic event.

Julia Willsie, a graduate student in James Clegg's laboratory, took embryos that had emerged from diapause and 'shocked them' into the nuclear p26 response. DNA replication and transcription are both switched off early in diapause, so she began to test the effect of p26 on transcription under a variety of stresses. Sure enough, transcription was depressed in the presence of stress-induced-p26, but the response varied depending on the type of stress, suggesting that p26 is not the only factor involved in switching the embryo 'off'.

Another clue came when she used confocal microscopy to visualise the localisation of p26 in nuclei. Willsie found high concentrations of p26 in stressed embryo nuclei, but she also found that a fraction of the unstressed control embryos had p26 localised in the nuclei. Coincidently, this was the same as the fraction of embryos that never hatch. Could p26 be part of a fundamental 'off switch' that can be reversed in diapause, but in a small fraction of cases, once thrown, can never be reset?

That leaves the next challenge to identify what interacts with p26 to produce such an incredible reaction. The control mechanisms that synchronize 4000 cells and simultaneously arrest their development are quite mind-boggling, and p26 may turn out to be a significant component of the complex molecular switching mechanism.

Fish Dinner (p. 2351)

Aquaculture is a relatively new form of cultivation that was developed in the early 1970s. Of course it has dramatically lowered the cost of many fish on the table. But there's no such thing as a free lunch, and the environmental costs are now becoming apparent.

Trout are naturally carnivorous, feeding on flies and larvae, so their metabolism is naturally geared towards a high protein diet. Artificial trout feed is synthesised from other fish and fish oils, which is economically costly, depletes natural fish populations and causes pollution. If the fish's diet could be switched in some way to include more carbohydrate, this would reduce the pressure on other fish stocks in the environment, and would also cut back on the ammonia that the fish excrete as a natural by-product of their diet. However, before you can fiddle with the fish's diet, it's best to know your enemy, which in this case is the way fish metabolise glucose.

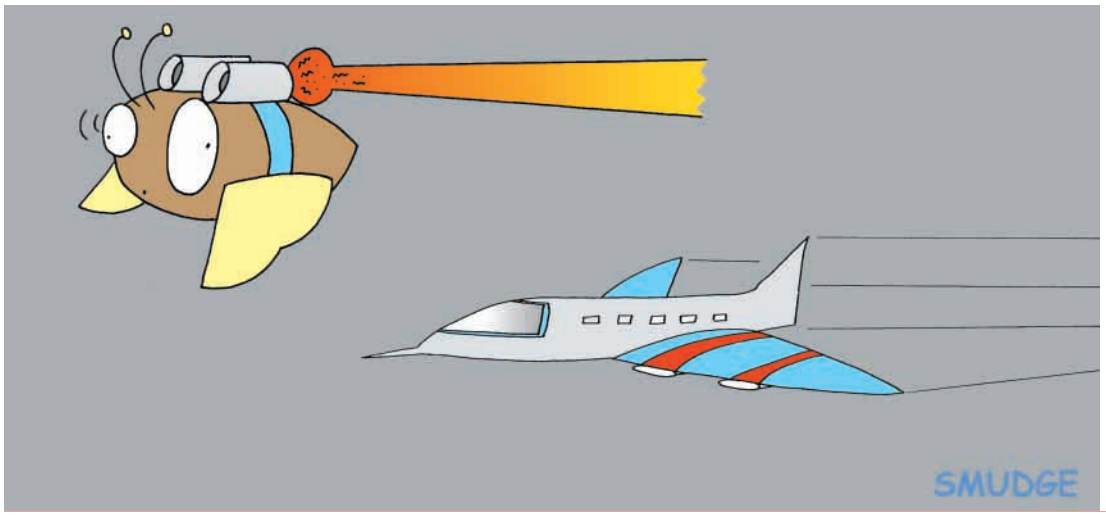
There are two possible reasons why fish don't benefit from a high carbohydrate diet. Either the fish can't store excess glucose because there's a problem with the metabolic pathways that produce glycogen or lipids, or, the liver doesn't turn off glucose synthesis when the diet is carbohydrate heavy. Stephán Panserat at the Laboratory of Fish Nutrition in France has confronted the problem by searching for the key enzymes in glucose metabolism with the aim of identifying the fish's Achilles heal.

He compared two groups of fish, which had been fed either 20 % carbohydrate or a normal diet, with fish that had been starved, and followed the expression of four critical hepatic proteins in two glucose pathways. By partially cloning the four key proteins, he was able to track the way the liver had responded to the different diets, and see how the fish's metabolism reacted to the carbohydrate-rich diet to see which aspect of carbohydrate metabolism is compromised in the fish.

What he found was that there seemed to be no problem with storing glucose. The three fish enzymes involved in glycolysis all behaved in exactly the same way as they do in mammals. But he did find that the levels of the enzyme fructose-1,6-bisphosphatase stayed high. This is a key enzyme in glucose synthesis (gluconeogenesis). So it appears that even if the fish has a rich source of glucose, the liver may continue to produce glucose unnecessarily. If the fish could down-regulate gluconeogenesis in response to carbohydrate in the diet, it would be able to survive on less protein and more carbohydrate.

So, the problem lies in the glucose synthesis branch of the fish's metabolism, and not in the way that the fish breaks down carbohydrate for storage as glycogen. This information is a crucial piece of the metabolic jigsaw that the aquaculture industry needs if it hopes to develop alternative plant-based feeds to clean up the world's fish farms.

Kathryn Phillips



TurboMoth

Wasserthal, L. T. (2001). Flight-Motor-Driven Respiratory Air Flow in the Hawkmoth *Manduca Sexta*. *J. Exp. Biol.* **204** 2209-2220.