Inside JEB 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

TURTLE ENERGY EXPENDITURE FROM ACCELERATION

Gliding through the warm south-western Indian Ocean, green turtles live a peaceful life feasting on seagrass meadows before returning to their deep coral reef homes. But their idyllic lifestyle is under threat from human activity; so understanding how these enigmatic creatures stay in tune with their environment is becoming increasingly important. 'If they don't balance their energy budget there is going to be a problem for the individual and later on at a population level,' explains Manfred Enstipp from the DEPE/CNRS Laboratory in Strasbourg, France. However, conventional methods for measuring energy expenditure may be unreliable in turtles, so Enstipp and Jean-Yves Georges decided to try another approach: measuring the animals' movements with accelerometers to estimate their energy use. First, the team had to discover whether there was a measurable relationship between the turtle's acceleration and their oxygen consumption in order to extrapolate the animals' energy use from their activity (p. 4010).

However, when Enstipp and his colleagues embarked on the study they weren't even sure that they could measure the turtle's oxygen consumption rate. 'They had to find this hole that is 40×40 cm to breathe so that we could measure the oxygen consumption rate,' explains Enstipp, but many of his colleagues thought that the freely swimming animals could not be trained to surface repeatedly at a single point in long channel.

Travelling to Stephane Ciccione's Kelonia Turtle Aquarium on Reunion Island, Enstipp isolated a 13 m-long swim channel in the turtles' enclosure and then, over a period of months, he and Benoit Gineste slowly covered the channel's surface with sections of metal fence until the turtles were content to surface and breathe at the respirometry dome, which covered the channel's only remaining open surface. Despite his colleagues' misgivings, Enstipp found that the turtles successfully learned to surface and breathe at the respirometry dome. Then, Enstipp velcroed a two-axis accelerometer onto a turtle's shell before recording her oxygen consumption and movements as she swam to and fro.

Returning to Reunion Island with Gineste, Myriam Milbergue and Virginie Plot in the summer and winter, Enstipp successfully measured the oxygen consumption rates of six adult turtles at temperatures ranging from 24.8 to 30.1°C while also measuring their acceleration patterns. But could he use the acceleration traces to accurately estimate the turtles' oxygen consumption?

Developing an equation to calculate the turtles' oxygen consumption based on their acceleration and the temperature of the surrounding water – which determines turtle metabolic rate – Enstipp calculated the turtles' oxygen consumption rates and compared them with the animals' measured oxygen consumption rates. They agreed well: the team could use the turtle's acceleration pattern to calculate their oxygen consumption, which they could use as a proxy for the animal's energy consumption.

But how does the captive turtle's activity level compare with that of free-ranging green turtles grazing their seagrass meadows? 'We are trying to apply this to Katia Ballorain's data from turtles foraging around Mayotte Island,' Enstipp reports. However, early evidence suggests that the captive turtles were no couch potatoes and were every bit as active as their free-ranging cousins, offering Enstipp and his colleagues hope that accelerometery could provide much needed measurements of green turtles' energy consumption in the wild.

10.1242/jeb.067074

Enstipp, M. R., Ciccione, S., Gineste, B., Milbergue, M., Ballorain, K., Ropert-Coudert, Y., Kato, A., Plot, V. and Georges, J.-Y. (2011). Energy expenditure of freely swimming adult green turtles (*Chelonia mydas*) and its link with body acceleration. *J. Exp. Biol.* **214**, 4010-4020.

Kathryn Knight

HONEY BEE LARVAE NEED IRS AND TOR TO BECOME QUEENS

All honey bee larvae are equal when they start out. But then, in a twist of fate, some develop into fertile queen bees, while others are relegated to a life of worker drudgery: and that twist is simply their diet. Larvae fed a nutritious gel – royal jelly – develop into queens, while larvae provided with a plainer diet develop into workers. However, the mechanism for this drastic diet-induced switch of fate wasn't clear. Adam Dolezal from Arizona State University, USA, explains that two metabolic pathways – the insulin-like signalling pathway and TOR (target of rapamycin) pathway – are known to sense the nutritional status of most creatures, adjusting their metabolism and development to make the most of an individual's diet. He adds that a key hormone – juvenile hormone – is also known to throw the switch from worker to queen development. So, Dolezal, his supervisor Gro Amdam and a team of collaborators from Arizona State University and Washington State University decided to find out whether these

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signalling pathways affect juvenile hormone levels in response to diet to turn workers into queens (p. 3977).

Knowing that both pathways are triggered by specific signalling proteins [insulin-like receptor substrate (IRS) and TOR], the team decided to inactivate IRS and TOR individually, and simultaneously, while feeding larvae on a diet of royal jelly. If the signalling pathways were carrying the message that the royal jelly-fed bees should develop into queens, then short circuiting the pathways should force the bees to develop into workers, despite the regal diet.

Dolezal and Navdeep Mutti became worker bee nurses to thousands of vulnerable honey bee larvae as they fed the insects a royal jelly diet laced with specially tailored double stranded mRNA molecules, to prevent the larvae from producing the IRS and/or TOR molecules and so inactivate the signalling pathways' responses to the diet. Then the team monitored how the larvae developed without the signalling pathways.

Teaming up with Florian Wolschin, Jasdeep Mutti and Kulvinder Gill, Dolezal and Nardeep Mutti found that the larvae developed into workers, despite their royal jelly diet. And when Dolezal monitored the larvae's juvenile hormone levels, instead of being high – which is what you would expect for larvae fed on royal jelly – the larvae had low levels of the hormone, which had forced them to develop into workers despite their diet.

Switching off the pathways prevented larvae on a royal jelly diet from producing juvenile hormone and developing as queens, but could the insects develop into queens if they received a juvenile hormone supplement? Sure enough, when Dolezal and Mutti gently applied juvenile hormone to the skins of royal jelly fed larvae that had lost insulin and/or TOR signalling, they developed into queens. So, the IRS and TOR signalling molecules are key links between the larvae's diet and their developmental fate.

However, Dolezal points out that another researcher, Masaki Kamakura, tested the link between diet and development at a different point in the insulin signalling pathway. Publishing his work in *Nature*, Kamakura found the opposite result: his royal jelly-fed larvae successfully developed into queens even though the signalling pathway had been short circuited at the signal's receptor. But Dolezal explains that the IRS trigger is also known to activate the epidermal growth factor (EGF) signalling pathway, in addition to the insulin pathway, by interacting with other receptors. The larvae could still develop into queens if the insulin receptor was inactivated because the IRS signal could trigger the alternative pathway, and the team is now keen to test the connection between IRS and EGF signalling in larval development.

10.1242/jeb.067108

Mutti, N. S., Dolezal, A. G., Wolschin, F., Mutti, J. S., Gill, K. S. and Amdam, G. V. (2011). IRS and TOR nutrient-signaling pathways act *via* juvenile hormone to influence honey bee caste fate. *J. Exp. Biol.* **214**, 3977-3984.

Kathryn Knight

DIAPAUSE TERMINATION PINPOINTED

Andrew Forbes

Some insects only have one annual crack at the whip. Emerging from a state of suspended animation during winter – known as diapause – many insects have a single season to successfully complete development and reproduce. And when your life-cycle is tightly synchronised with other seasonal events, such as fruit production, accurate timing is essential. Yet little was known about how pupae reactivate development when they terminate diapause ready for a new season.

According to Gregory Ragland from the University of Florida, USA, larvae of the apple maggot fly (*Rhagoletis pomonella*) are a major apple pest in the US. However, *R. pomonella* larvae infested indigenous hawthorn fruits – which fruit later than apples – long before settlers brought apples to North America, so the flies that switched host had to evolve rapidly to adjust when they emerge from diapause to catch early fruiting apples. Knowing that *R. pomonella* is studied by scientists intrigued by rapid species evolution, Ragland, Jeffery Feder, Stewart Berlocher and Daniel Hahn decided out find out how the *R. pomonella* population that persists in attacking hawthorn fruits initiate emergence from diapause (p. 3948).

Collecting maggot infested hawthorn fruits from a field in Michigan, Ragland and Hahn transported them to Florida and collected the larvae surfacing from the fruit. Next, the duo simulated the onset of winter by cooling the larvae to 4°C as they transformed into pupae and entered diapause. Then, after 20 weeks in the cold, the team warmed the pupae to 24°C and monitored their metabolic rate to track when it rose and they began to emerge from diapause. Collecting pupae before their metabolic rate began rising, when their metabolic rate had risen by 40% from the diapause level over 24 h, and 48 h after their metabolic rate began to rise, the team analysed how the insect's gene expression patterns changed using a custom-built gene chip containing between 7000 and 8000 unique *R. pomonella* genes. The team compared how the expression of groups of genes changed over the first 48 h after the larvae's metabolism began rising to find out how they terminate diapause and reinitiate development and growth.

'The punch line of the analysis is that almost all the patterns had this large change in gene expression that happened right when we had the metabolic rate increase,' says Ragland. And when Ragland and Scott Egan took a closer look at the genes that were strongly activated, they found several genes involved in cell cycle control, which regulates when cells divide. 'The cell cycle is arrested during diapause and we see marked changes in activity in some of the important cyclins and cyclin-dependent kinases [that regulate the cell cycle] concordant with the up-tick in metabolism. That suggests to us that they are getting ready for, or they are going through, mitosis [cell division] at that point and it is roughly concordant with the increase in metabolic rate, so we feel we have pinpointed pretty well when development starts up and diapause termination is complete,' says Ragland.

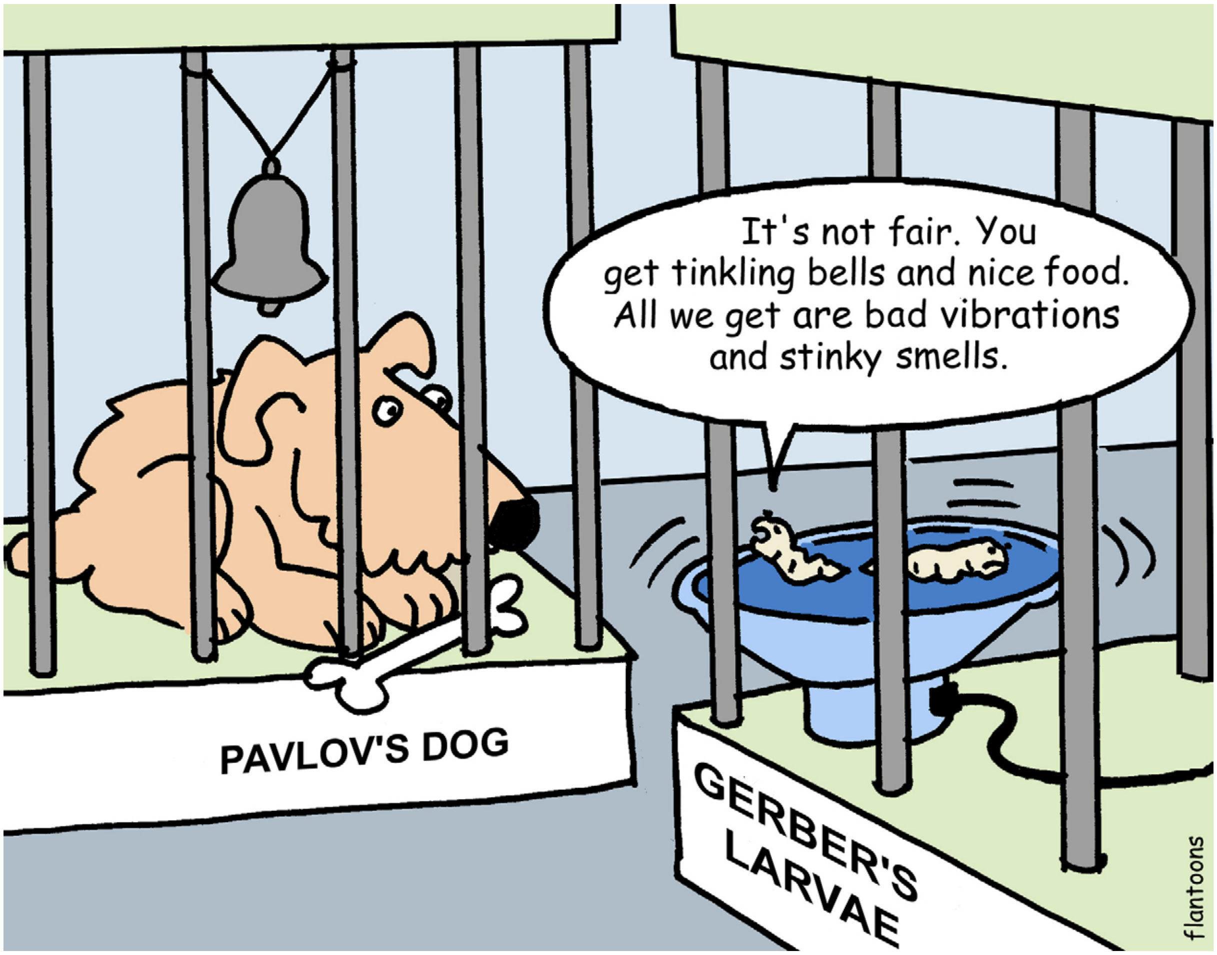
The team also found changes in heat shock protein expression, metabolic gene expression, genes involved in development, and genes that are known to participate in diapause termination in other organisms. They are now keen to find genes that have different regulation patterns in the late emerging hawthorn maggots and early emerging apple maggots to identify other key components of the diapause termination process.

10.1242/jeb.067090

Ragland, G. J., Egan, S. P., Feder, J. L., Berlocher, S. H. and Hahn, D. A. (2011). Developmental trajectories of gene expression reveal candidates for diapause termination: a key life-history transition in the apple maggot fly *Rhagoletis pomonella. J. Exp. Biol.* **214**, 3948-3959.

Kathryn Knight

LARVAE ASSOCIATE BAD VIBRATIONS WITH ODOURS



Understanding the neural basis of memory and learning is one of the Holy Grails of neurobiology, but untangling convoluted memory networks in human brains is currently impossible. So neurobiologists have turned their attention to animals that learn and form memories in much simpler brains. Bertram Gerber and colleagues from Germany explain that *Drosophila* larvae can learn to associate odours with punishment – unpleasant tastes and mild electric shocks – but no one had tested whether they could associate odours with disturbing vibrations. Given that vibration can be more finely controlled than other aversive stimulants, the team decided to find out whether the larvae could be trained to associate disagreeable vibrations with specific odours (p. 3897).

Training 50 larvae in a Petri dish by exposing them to 200 ms bursts – a buzz – of 100 Hz vibration from a loudspeaker in the presence of 1-octanol odour and then exposing them to *n*-amyl acetate with the loudspeaker turned off, the team then tested which odour the larvae avoided in the presence of the vibration threat: the larvae wriggled away from the 1-octanol odour. And when the team repeated the experiments, this time associating the vibration with *n*-amyl acetate odour and no vibration with 1-octanol, the vibrated larvae avoided n-amyl acetate when presented with a choice.

After characterising the larvae's behaviour as an escape response, the team also found that the strength of the association between the vibration and odour increased when they increased the number of buzzes and the length of the training period. However, the team point out that the larvae only take evasive action when under threat from the vibration, and they say, 'Aversive memories are behaviourly expressed in the presence of punishment but not in its absence, and… are embedded into a conditioned "escape routine" which is employed only when escape is warranted.'

10.1242/jeb.067082

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