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

SIBLINGS SET THE SOCIAL SCENE

Brush-turkeys probably won't win the 'most doting parent' prize. Abandoning their newly laid eggs to develop in enormous compost heap incubators, the parents return to the Australian bush ready to resume their solitary existence. So brush-turkey chicks must be resourceful from the start, scratching around without a mother's example to guide them; 'brush-turkey chicks are probably the most precocial chicks there are' explains Ann Göth. But it wasn't clear whether these abandoned hatchlings had the same social skills as other precocial chicks, which imprint on their mothers. Göth wondered whether brush-turkey chicks could imprint on other brush-turkey chicks instead. Working with Christopher Evans, she decided to test whether newly hatched brush-turkey chicks could recognise each other, and what attracts a hatchling to another chick shortly after it emerges from its decomposing incubator (p. 2199).

But getting hold of brush-turkey eggs was far from straightforward. Göth had to brave leeches as she manually excavated the bird's enormous leaf litter incubators and transferred the eggs to soil incubators in the lab at Macquarie University. Having got some eggs, Göth also knew she had to keep the newly hatched chick's contented and unaware of their unnatural surroundings when they hatched, which meant constructing large net aviaries in an area of native forest on the University's campus.

Having ensured that the chicks' Macquarie home was as natural as possible, Göth had one final test to do before putting newly hatched chicks through their social paces. The chick robots, which she and Evans had built, looked fine to human eyes, but would the real chicks fall for their doubles? Tentatively she tested five newly hatched youngsters' reactions to the pecking impostors, and was delighted when all of the chicks began acting socially, copying the robot's pecking action. Happy that the chicks were none the wiser about the chick-doubles, Göth was ready to test their preferences.

Suspecting that the chicks would be most attracted to pecking actions, Göth allowed the newly hatched youngsters to choose between either a pecking robot or a robot involved in some other activity. But no matter which choice the chicks were offered, they always seemed to prefer spending time with the pecking robot chick, even trying to steal the food that the automated model appeared to dine on. The chicks were very sociable, despite their parents antisocial reputation.

Göth wondered whether any other aspects of a brush-turkey chick's appearance attracted the youngsters and found that UV wavelengths reflected from brush-turkey's legs and beak were essential if the chick was to mimic its pecking actions. The team suspect that the chick's spectral properties could form part of a secret signal visible only to the UV sensitive chicks, but invisible to mammalian predators and airborne hunters.

So even though they don't have a mother to imprint on, newly hatched brush-turkey chicks seem to have well developed social responses, despite the lack of parental guidance.

10.1242/jeb.01062

Göth, A. and Evans, C. S. (2004). Social responses without early experience: Australian brush-turkey chicks use specific visual cues to aggregate with conspecifics. *J. Exp. Biol.* **207**, 2199-2208.

ARE MEMBRANES PACEMAKERS?

Since the early 19th century, scientists have puzzled over the factors that regulate metabolic rate. At first people suspected that an animal's metabolic rate was directly proportional to its surface area, but it became clear more recently that a creature's metabolic rate was proportional to a value close to its mass raised to the power 0.75. Although the factors that govern this convenient relationship still aren't known, Paul Else and Tony Hulbert in Wollongong, Australia, suggest that an animal's lipid membrane composition might act as a metabolic pacemaker to regulate their metabolic rate. Having previously found a strong relationship between the degree of membrane polyunsaturation and the metabolic rate of mammals, Hulbert was curious to find whether birds' metabolic rates also correlate with their lipid constituents. After all, both class of vertebrate evolved endothermy independently, so if both vertebrate's membrane compositions were highly correlated with their metabolic rates, maybe membrane composition would be the essential regulator that sets all endotherm's metabolic rates. Knowing that larger birds' lipid membranes tended to be composed of monounsaturated fatty acids, while smaller birds with high metabolic rates produce membranes with high levels of polyunsaturated fatty acids, Else and Hulbert have investigated the metabolic rate of hepatocytes from birds ranging in size from 13 g up to 35 kg, and found that small birds have higher oxygen consumption rates than large birds (p. 2305).

Fortunately, collecting birds that covered the size range the team needed wasn't too difficult; Australia boasts some of the largest birds on the planet. Else, Hulbert and Martin Brand bought some species from local suppliers and trapped others from the wild. But the emus were much less cooperative. Hulbert had to convince the huge birds to sit down by leaping on their backs before they could be safely secured in a wool sac and driven to Wollongong. Only then were the team were ready to start measuring tissue metabolic rates.

Extracting isolated hepatocytes from liver samples, they found that the larger birds' mass-specific metabolic rates were lower than the smaller species. The team also measured the relative sizes of the birds' livers and found that livers from the smaller species were proportionately larger than those from the goose and emu. Not only was their metabolic rate higher, but the tissue comprised a larger fraction of their body mass, contributing to the tiny birds' high metabolic rate.

Next the team wondered which cellular metabolic processes were increased to raise the smaller birds' metabolic rates. By systematically inhibiting mitochondrial ATP synthesis and the mitochondria's electron transport chain, they measured the hepatocytes' oxygen consumption rate and found that the cells always used the same proportion of energy for ATP production, proton leak, and other cellular processes, regardless of the animal's size. Like mammals, small birds increase all of their metabolic processes to raise their metabolic rate.

Although this work goes some way to support Else and Hulbert's metabolic pacemaker theory, they emphasise that this is one aspect of a much larger investigation into the metabolic effects of membrane composition, which may one day explain one of the most fundamental relationships in physiology.

10.1242/jeb.01061

Else, P. L., Brand, M. D., Turner, N. and Hulbert, A. J. (2004). Respiration rate of hepatocytes varies with body mass in birds. *J. Exp. Biol.* **207**, 2305-2311.

EGGS SUFFER IN HEAT

Art Woods is fascinated by tobacco hornworms. And he's luckier than most, he's seen them in their natural environment, the North American Sonoran desert, where they are far more intriguing than the dull insects reared in laboratories. Wondering how the insects extract all of the essential elements they require from their harsh environment, Woods has turned his attention to their eggs. He was puzzled by the problems insect eggs face supplying the developing embryo with adequate oxygen, while remaining sufficiently watertight to protect the egg from dehydration. Woods wondered whether the environmental temperature might affect oxygen diffusion into the egg and limit the developing embryo's access to oxygen at temperatures experienced by the embryos on a typical Sonoran day (p. 2267).

But first, Woods needed to discover whether the developing embryos were sensitive to oxygen availability, as adults seem unaffected by varying oxygen levels. Maybe the embryos' development would be unaffected. Working with the team in the local chemistry workshop, Woods designed a Plexiglas block where he could control the partial pressure of oxygen in various egg-filled chambers while filming their development. Monitoring the eggs' development at oxygen concentrations ranging from 21% down to 9%, and temperatures from 22 up to 32°C, the results were dramatic. Woods watched the hatching events move through his Plexiglas box like a wave, with the warmest and most highly oxygenated eggs hatching first, while eggs incubated at lower oxygen concentrations and temperatures hatched later. But when he looked closely to see if there was an interaction between temperature and oxygen that affected the insects' development, he failed to find any. Would he see a strong interaction by looking at other metabolic indicators over a shorter period?

Woods decided to measure the eggs' carbon dioxide production rates to see if temperature and oxygen supply might affect their metabolic rate. But it took another trip to the chemistry workshop to crack the problem of measuring the tiny amounts of carbon dioxide produced by clutches of 40 eggs. Woods was now ready measure the eggs' carbon dioxide production rates over oxygen concentrations ranging from 5–50%.

This time he found a strong interaction between temperature and oxygen levels; the eggs' carbon dioxide production rates were far more sensitive to oxygen availability at higher temperatures. And when Woods raised the oxygen level to 50%, the egg's metabolic rate rose still further. It seemed that the oxygen supply within the egg was limited even at naturally occurring temperatures, and as the temperature rose, the limitation became worse.

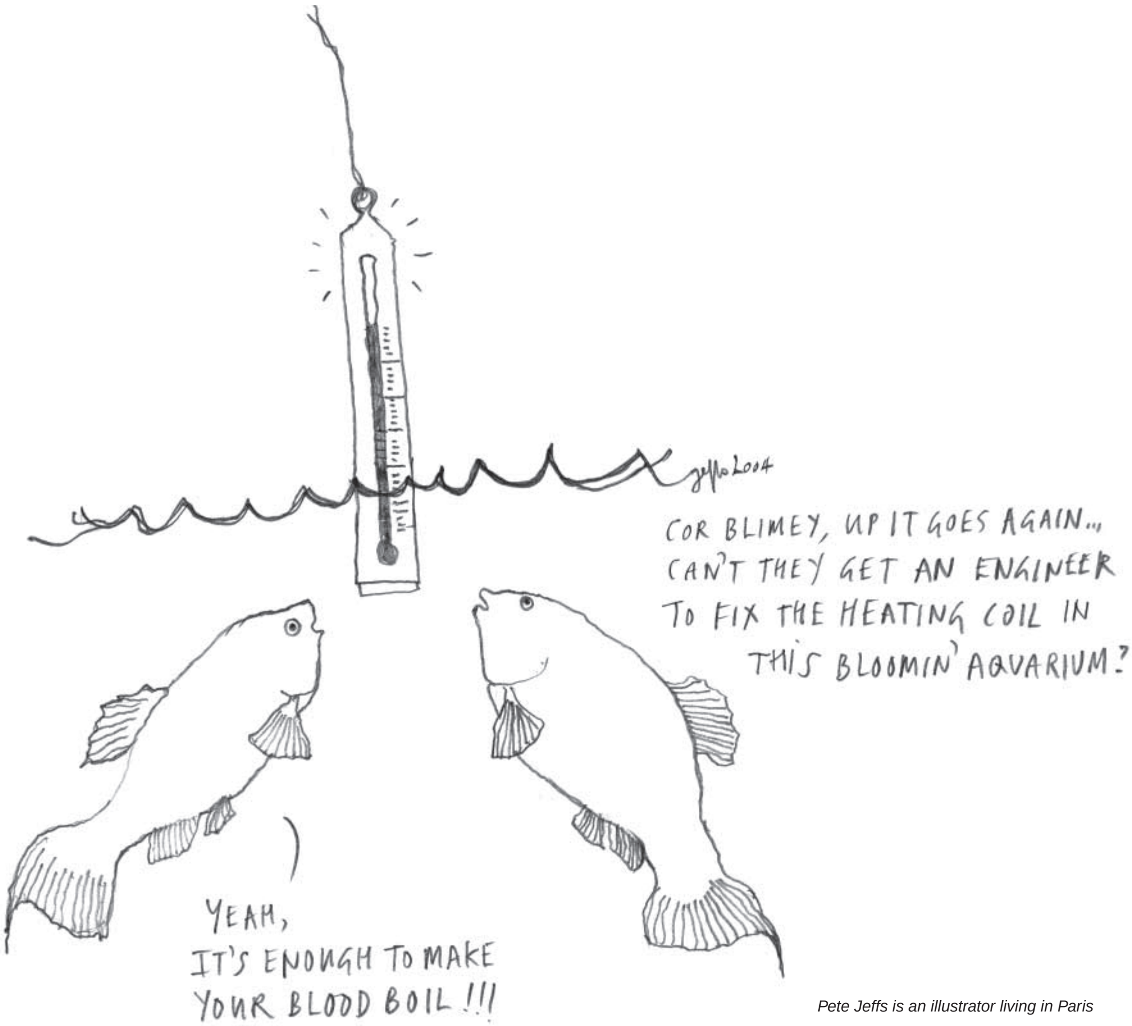
But would this be reflected in the amount of oxygen inside the egg's tissues? Woods measured the oxygen gradient within the eggs by gently inserting an oxygen electrode, and found that the oxygen concentrations dropped dramatically, plummeting to 7% of air saturated levels at the egg's core. 'This is really neat, that such a small piece of tissue could have problems with oxygen supply' says Woods.

Having found that tobacco hornworm's eggs appear to be oxygen limited, Woods is keen to investigate how the eggs respond to the natural temperature fluctuations they experience while incubating on leaves in the desert, and the challenges they face supplying sufficient oxygen to the embryo within.

10.1242/jeb.01063

Woods, H. A. and Hill, R. I. (2004). Temperature-dependent oxygen limitation in insect eggs. *J. Exp. Biol.* **207**, 2267-2276.

WHEN THE GOING GETS HOT...



Pete Jeffs is an illustrator living in Paris

Some days, choosing the right clothes isn't easy. It might be freezing when you leave home, but once the sun's beating down, some early morning sartorial choices might not seem so wise. And if we find diurnal temperature fluctuations stressful, spare a thought for the tiny annual killifish, trapped in its coastal desert ponds; they routinely weather 20°C temperature fluctuations. Jason Podrabsky and George Somero are fascinated by the physiological adaptations that allow creatures to survive and thrive under extreme environmental variations. Curious how the little fish's gene expression patterns change under several thermal conditions, they designed a novel gene chip, coated with 4992 killifish liver genes, to find out how the fish faired after acclimation at 20°, 26° and 37°C, and a daily temperature cycle ranging from 20–37°C over a 2 week period (p. 2237).

Using the custom made gene chip to compare mRNA samples from fish exposed to one of the four temperature regimes, with mRNA from control fish, Podrabsky and Somero found that 540 genes changed their expression patterns significantly, with different patterns in the fish exposed to a daily temperature cycle compared with fish acclimated to constant temperatures.

Grouping the genes according to their cellular functions, Podrabsky found that molecular chaperones, membrane structure and solute transporters were all affected by the temperature treatments, as well as various metabolic genes, cytoskeletal genes, genes involved in protein turnover, immune response and cell growth. Some of the genes which responded to temperature haven't even been assigned functions yet, and Podrabsky is excited by the possibility of discovering new temperature sensitive factors and pathways.

But Podrabsky is most intrigued by a single transcriptional regulator, HMGB1, which decreases its transcriptional levels tenfold as the temperature rises daily. Podrabsky suspects 'HMGB1 protein may be … a highly sensitive temperature sensor' by causing a 'global change in the rate of transcription'.

10.1242/jeb.01060

Podrabsky, J. E. and Somero, G. N. (2004). Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* **207**, 2237-2254.

Kathryn Phillips
kathryn@biologists.com