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

GROWING EMU TAKE THE STRAIN

Picture by Russell Main

If internal skeletons didn't adapt to the ever larger forces exerted on them as organisms grow, they would eventually break under the increasing stresses and strains. But fortunately bones change in response to an organism's growth and movement. Having studied how the bones of goats change as they grow, Russell Main and Andrew Biewener turned their attention to a model of two-legged locomotion, the emu. Emu are ideal creatures to study because they increase in mass by around 65 times as they grow from 25 cm tall hatchlings weighing 0.74 kg to 55 kg adults up to 2 m tall. 'The goal was to examine growth and development in the emu hind limb skeleton and see how it is affected by changes in locomotor mechanics and body mass during development', says Main (p. 2676).

To monitor the movements of the limb joints and forces generated by different sized emu, the team placed external markers on the leg joints and monitored their movements with a video camera as the emu ran down a runway with force plates embedded in the floor. They found that both big and small emu walked or ran in very similar ways, and that the maximum force they generated as their feet hit the ground remained very similar in proportion to their body weight.

Having shown that how emu run doesn't change much as they grow bigger, the team attached strain gauges to the two largest leg bones: the femur and the tibiotarsus. During a delicate operation, the team attached either a single strain gauge, pointing down the length of an emu's bones, or a 'rosette' of three strain gauges arranged on top of each other, each at a 45° angle to the one above it, which could measure strain in three different directions at once. Wires from the strain gauges travelled under the skin to an external cable at the hip.

Following surgery, the emu still generated the same forces with their operated legs as they ran on a treadmill. Concentrating on the strain patterns the gauges recorded, the team found that at each of the sites they measured in both bones, the patterns of strain were similar in different sized emu. However the amount of strain differed in some parts of the bones with age. The strain over the front and back surfaces of the femur and over the tibiotarsus increased as emu grew. This means that as emu get bigger, their bones experience relatively larger strains. The team found that the leg bones stayed a similar size and shape relative to increasing body mass, rather than becoming relatively more robust as they grow, which might otherwise be expected to support the extra strain.

Other measurements that the team made on the bones gave them some clues as to how the emu partly deal with these increasing strains as they grow. They found that as the emu grew both leg bones became straighter, which decreases the strains that a bone experiences. They also found that the bone mineral content increased from 50% in small emu to 70% in large emu, which makes the bones much stronger and better able to support an emu's increasing bulk.

10.1242/jeb.009605

Main, R. P. and Biewener, A. A. (2007). Skeletal strain patterns and growth in the emu hindlimb during ontogeny. *J. Exp. Biol.* **210**, 2676-2690.

GOOD VIBRATIONS

For years, scientists assumed that because moth ears have a simple structure, their mechanical response to sound might also be quite simple, despite the fact that they can code complicated sounds. But appearances can be deceptive: locusts' ears are also structurally simple but respond to sound in a more complex way than previously thought. So James Windmill and his colleagues James Fullard and Daniel Robert decided to investigate in detail how the ears of four noctuid moth species physically respond to sounds (p. 2637).

Because moths are preyed on by bats, researchers have intensively studied their behavioural and neural response to bats' ultrasonic calls, to find out what moths can

hear and how they could avoid predation. But relatively little is known about how the ear actually moves to code sounds. A moth's ear consists of two regions of membrane, called the conjunctivum and the tympanum, that are separated by a piece of cuticle. In the centre of the tympanum there is a small circular opaque zone, where the receptor cells that pick up sound vibrations attach, surrounded by a transparent and thinner region of membrane. Researchers had assumed that the whole tympanum would move much like the skin of a drum.

To find out how the conjunctivum and the tympanum move during hearing, the team used a technique called microscanning laser Doppler vibrometry. They glued captured moths upside down onto a stand, and focussed a laser beam at a set frequency onto the moths' ears as they played them sounds within their hearing range. As they moved the laser beam over the ear's surface, they recorded the frequency of the laser beam as it bounced off the membranes. Because vibrations in the membrane affect the laser beam's frequency, the team could calculate the ear's movements according to the changing frequency of the returning laser beam.

They found that the pattern of the membranes' mechanical responses to sound were similar in all four moth species, although each species responded best to a different set of ultrasonic frequencies. Comparing the response of the conjunctivum and the tympanum, they found that they moved in anti-phase with each other. The conjunctivum moved more at lower sound frequencies, although what role these movements play in hearing is still a mystery, since the conjunctivum doesn't have any receptor cells attached to it to pick up the vibrations.

Focussing their attention on the tympanum, the team found that it responded best to higher frequencies. Rather than behaving like the skin of a drum, the team discovered that the central opaque zone vibrated in response to sound, but that the surrounding circular zone vibrated very little. The team suspect that this response could somehow focus the sound onto the receptor cells.

They also found that the tympanum moved as little as 100 pm (a minuscule 0.0000001 mm) in response to sounds just above the moths' auditory threshold. To confirm that these tiny movements caused the receptor cells and auditory neurons to respond, they recorded from the auditory neurons, finding that they did respond to 100 pm movements. Not only does this show that the moth ear is incredibly sensitive, but that it is also far more complex than previously thought.

10.1242/jeb.009613

Windmill, J. F. C., Fullard, J. H. and Robert, D. (2007). Mechanics of a 'simple' ear: tympanal vibrations in noctuid moths. *J. Exp. Biol.* **210**, 2637-2648.

BREATHE IN…

Northern elephant seals (*Mirounga angustirostris*) are most at home in the water, plumbing the depths during their extensive dives and holding their breath for an hour or more, tolerating conditions that would damage humans irreversibly. The seals only emerge onto land for 2–4 months of the year to breed and moult. When asleep on land they show long natural breath-holds called apneas, which last up to 25 min. These apneas probably occur to conserve energy and water since the seals fast while they are on land, and studying them might give scientists clues as to how seals manage their oxygen stores when they hold their breath. To find out more, Torre Stockard, Paul Ponganis and colleagues monitored nine juvenile elephant seals as they slept (p. 2607).

To measure oxygen levels in the blood, the team inserted catheters into the arteries and veins supplying the major internal organs and took blood samples during normal breathing and at regular intervals during apnea. To find out how much of a blood oxygen store the seals had available before apnea, they measured the percentage of the blood that is made up of red blood cells, blood volume, haemoglobin content and its saturation with oxygen. They found that the seals could store around 52 ml O_2 in their blood per kilo of body mass, whereas humans can only hold around 10 ml O_2 per kilo in their blood, showing that large oxygen stores contribute to seals' diving prowess.

When the seals nodded off and started showing apneas, which lasted between 3 and 11 min, they used up oxygen at a rate of 2-2.3 ml oxygen per 100 ml blood each minute, which is a similar rate to normal conditions. By the end of 7–9 min apneas, the seals had significantly depleted their blood O_2 store from around 26 ml per 100 ml blood, to around 7 ml per 100 ml blood, a very low level that humans couldn't tolerate.

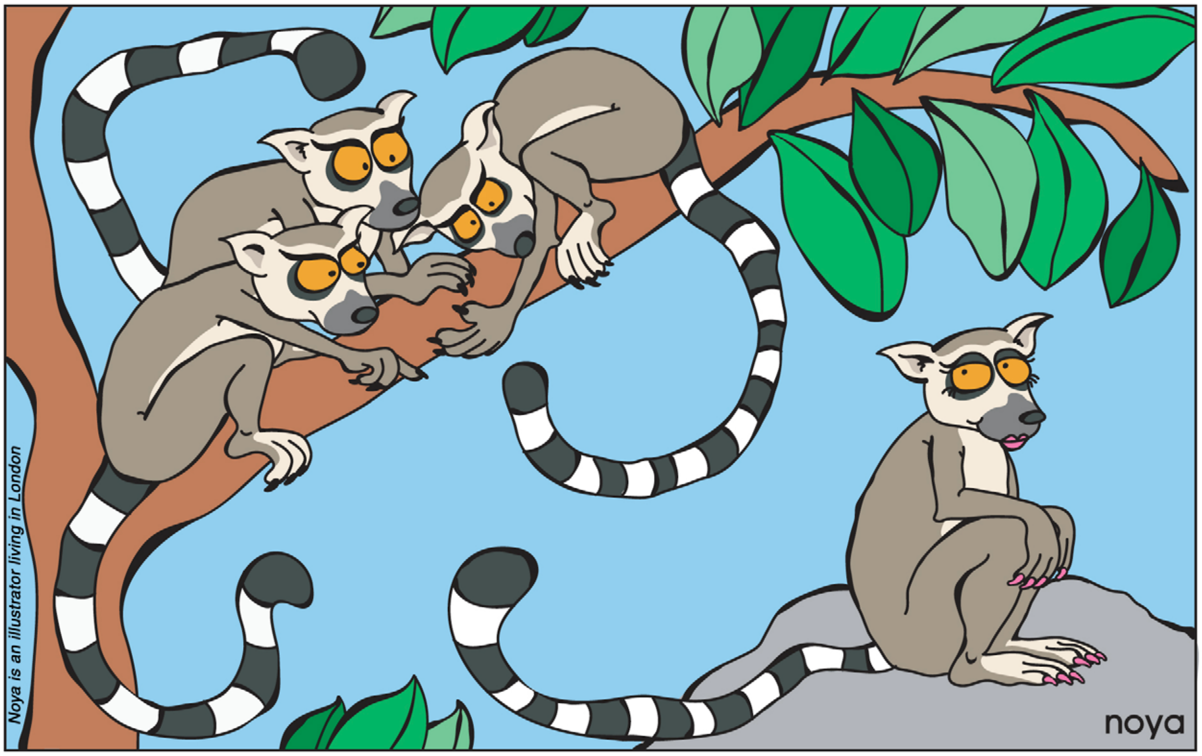
To find out how much oxygen can be transferred from blood to tissue during apnea, the team measured oxygen partial pressure, which is the pressure exerted by an individual gas in a gas mixture, or in solution. The lower the partial pressure gradient, the slower the transfer to tissues. Although the oxygen partial pressure varied greatly during normal breathing, between 34 and 108 mmHg, partial pressure dropped in all the seals during apnea, and the lowest recorded arterial value was 18 mmHg. Humans black out at an oxygen partial pressure of around 25-30 mmHg, showing that the seals are able to squeeze the most out of their oxygen store to get the gas to the tissues that need it, and tolerate very low oxygen levels without any ill-effects.

The seals used up around 56% of their oxygen stores during a typical 7 min apnea, but one animal pushed the limit and used up nearly 88% of its store during an 11 min apnea. Despite the oxygen stores getting very low in some cases, none of the seals showed evidence of anaerobic metabolism, since there wasn't a build up of anaerobic by-product lactate in the blood. A small decrease in blood pH was accounted for by carbon dioxide accumulating in the blood. 'Their breath-holding is very efficient', says Stockard. 'Diving is the norm for these seals, so next we're going to find out what's really happening in diving animals'.

10.1242/jeb.009621

Stockard, T. K., Levenson, D. H., Berg, L., Fransioli, J. R., Baranov, E. A. and Ponganis, P. J. (2007). Blood oxygen depletion during rest-associated apneas of northern elephant seals (*Mirounga angustirostris*). *J. Exp. Biol.* **210**, 2607-2617.

FEMALE LEMURS SNIFF OUT THE COMPETITION



To a ring-tailed lemur (*Lemur catta*), smell is all important, and they not only use their genital secretions to warn rival lemurs not to trespass on their territory, but also to inform each other about their sexual status. Elisabetta Palagi and Leonardo Dapporto wanted to know if lemurs could distinguish the scents of individual females from different groups that compete for territory (p. 2700).

Analysing the chemical components of individual female's scent, the team found that each female's scent had a unique chemical signature, which didn't change over years, seasons, or reproductive cycles. They then showed that both male and female lemurs could tell filter paper soaked with secretions apart from clean paper: the lemurs indicated their preference for the scent-soaked paper by sniffing it for longer. Having shown that each female's scent is unique, and that both sexes can distinguish a female scent, the team tested how lemurs respond to scents from different females. While males did not distinguish between any of the scents tested, females investigated the scent from the more serious territorial rival the longer. Given the choice between a familiar female scent from their own group and an unfamiliar female scent, the females investigated the unfamiliar odour for longer, because they see scents from unfamiliar females as more of a threat.

Replacing the odour of the familiar female with the scent of a female competitor from a rival group, however, caused the females to prefer the competitor's scent over the unfamiliar scent. This is because the rival female is a known competitor for territory, so is a more serious threat than an unknown lemur. In this particular experiment, males likely see all females as potential mates, so investigated all scents equally, while females are sniffing out potential rivals.

10.1242/jeb.009639

Palagi, E. and Dapporto, L. (2007). Females do it better. Individual recognition experiments reveal sexual dimorphism in *Lemur catta* (Linnaeus 1758) olfactory motivation and territorial defence. *J. Exp. Biol.* **210**, 2700-2705.

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