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INSIDE JEB

Transparent larvae hide eyes behind reflections

Pseudosquillana thomassini larva with blue ventral eyeshine. Photo credit: K. D. Feller.

Becoming invisible is probably the ultimate form of camouflage: you don't just blend in, the background shows through you. And this strategy is not as uncommon as you might think. Kathryn Feller, from the University of Maryland Baltimore County, USA, explains that the larval life stages of many marine species are transparent. However, there is one part of the anatomy that most creatures cannot make transparent. Feller explains that the animals with compound eyes have to shield each individual eye unit with an opaque pigment to prevent light leaking between adjacent eye structures. This could blow the larva's cover and poses the question, how do larvae disguise their conspicuous eyes? Many aquatic species use reflectors on scales to reduce their contrast with the background, so when Thomas Cronin told Feller that the eyes of tiny mantis shrimp larvae shone when caught in light, she wondered whether the transparent larvae were hiding their opaque compound eyes behind a reflection (p. 3263).

Feller headed south to the Lizard Island Research Station on Australia's Great Barrier Reef, where she could wade into the tropical waters at night to lure tiny mantis shrimp larvae into her net, ready to investigate their microscopic eyes. She illuminated the larvae's eyes with white light back in the lab, and recalls that the display was dazzling: 'The whole sphere of the retina at the centre of the eye reflects this sparkly blue-green light; it's quite brilliant.'

Measuring the spectrum of the reflected light – known as eyeshine – Feller realised that the minute mirrors in the eyes of *Pseudosquillana richeri* and a *Harpiosquilla* larva only reflected blue-green light: 'They produced very discrete peaks in that region of the spectrum', she says. However, when she investigated the eyes of *Pullosquilla thomassini*, she was amazed to see that the upper region of the eye produced green reflections, while eyeshine from the lower portion of the eye was blue. 'We suspect that it is something similar to counter shading; perhaps the dorsal part of the eye is held against background that is greenish and the ventral part of the eye is more bluish', Feller suggests.

But how well would these reflections conceal the larvae's conspicuous eyes? Donning SCUBA gear, Feller took some of the larvae back to the ocean so that she could photograph their eyes against the natural background. 'It was very labour intensive to get the *in situ* images', recalls Feller, adding that it took a day to collect shots of each larva from different directions at various depths and times of day.

Back in the lab, Feller subtracted the intensity of the background from the eyeshine and calculated the eye's contrast to see how well the eyes blended in, and admits that she was amazed to see that there was virtually no contrast between the eye reflections and the surrounding lighting environment. 'Larval eyeshine does a really nice job, I didn't expect it to do as good a job as it does, that's for sure', Feller chuckles. And when she compared the spectrum of the eyeshine with the spectrum of the surrounding environment, they were a tight match. 'The peak wavelength that is reflected off the eye is the same as the peak wavelengths that are available in the environment', says Feller, adding that larvae found in Atlantic waters produce eyeshine with a completely different spectral range that is probably tuned to their light environment too.

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Feller, K. D. and Cronin, T. W. (2014). Hiding opaque eyes in transparent organisms: a potential role for larval eyeshine in stomatopod crustaceans. *J. Exp. Biol.* **217**, 3263-3273.

Kathryn Knight

Lunar explorers will walk at higher speeds than thought

John De Witt running on a treadmill in lunar gravity. Photo credit: NASA.

Anyone who has seen the movies of Neil Armstrong's first bounding steps on the moon couldn't fail to be intrigued by his unusual walking style. But, contrary to popular belief, the astronaut's peculiar walk was not the result of low gravity. Wyle Science, Engineering and Technology scientist John De Witt explains that the early space suits were not designed for walking, so the astronauts adapted their movements to the restrictions of the suit. Michael Gernhardt, the head of NASA's Extravehicular Activity Physiology, Systems and Performance Project, wants to learn more about how humans move in low gravity, including the speed at which we break from a walk into a run, to design a modern space suit that permits freer movement. However, the only way to test the effects of true lunar gravity on our movements while based on earth is to hop aboard NASA's adapted DC-9 aircraft – which reduces the gravity on board by performing swooping parabolic flights – and get running (p. 3200).

De Witt and colleagues Brent Edwards, Melissa Scott-Pandorf and Jason Norcross recruited three astronauts and five other registered test subjects that could tolerate the discomfort of the aircraft's bucking flight to test their

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running. 'There is some unpleasantness,' recalls De Witt, adding, 'if you get sick you're done…. We wanted to be sure we had people that were used to flying.' Once the subjects were airborne, the team only had 20 s during each roller-coaster cycle – when the gravity on-board fell to one-sixth of that on Earth – when they could test the runner's walking and running styles on a treadmill as the volunteers shifted over a range of speeds from 0.67 to 2 $m\,s^{-1}$. However, the experiments ran smoothly after the first few parabolas once the team had settled into a routine.

Back on the ground, De Witt and colleagues analysed the speed at which the walkers gently transitioned into a run. 'Running is defined as a period of time with both feet off the ground', explains De Witt, adding that the walk to run transition was expected to occur at 0.8 $m\,s^{-1}$ in lunar gravity, based on theoretical calculations. However, when the team calculated the transition speed from their experiments, they were in for a surprise: 'The average was 1.4 $m\,s^{-1}$', recalls De Witt.

'This difference is, to me, the most interesting part of the experiment; to try to figure out why we got these numbers', says De Witt, who suggests that the acceleration forces generated by the counter-swinging arms and legs could account for the shift in transition speed. 'What I think ends up happening is that even though the atmosphere is lunar gravity, the effective gravity on our system is lunar gravity plus the forces generated by our swinging arms and legs', says De Witt. He explains that this arm-and-leg swinging effect probably happens here on Earth too, but the forces generated by the swinging limbs are negligible relative to our gravity. However, he suspects that they are more significant in weaker lunar gravity, saying, 'They contribute more to the gravity keeping you attached to the ground.'

De Witt also adds that the higher transition value is not without precedent. He explains that scientists on Earth have simulated lunar gravity by supporting five-sixths of a runner's weight in a sling, and the athletes also transitioned from a walk to a run at speeds of around 1.4 $m\,s^{-1}$. 'This tells researchers [that] what they have in the lab, which is a fraction of the cost of the airplane, is probably adequate at giving you the information you need', he says.

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De Witt, J. K., Edwards, W. B., Scott-Pandorf, M. M., Norcross, J. R. and Gernhardt, M. L. (2014). The preferred walk to run transition speed in actual lunar gravity. *J. Exp. Biol.* **217**, 3200-3203.

Kathryn Knight

Parental lifestyle influenced eggshell evolution

Red-throated diver (*Gavia stellata*) egg and Slavonian grebe (*Podiceps auritus*) eggs. Photo credit: G. Maurer.

The next time you make an omelette, take a moment to think about the shell that you have just destroyed. In addition to protecting the young within, the remarkable structure provides calcium for development as well as permitting essential gases to leave and enter. 'The avian eggshell is a complex bioceramic', says Steven Portugal from The Royal Veterinary College, UK. But the structure can also teach us about the factors that influence biological diversity. Portugal and a team of international colleagues explain that eggs are laid under a wide range of different circumstances – in locations from burrows and cliffs to high in trees and low on the ground – to parents whose lifestyles range from the semi-aquatic to those that are almost permanently on the wing, and many of these factors could dramatically affect the passage of water vapour through the shell. But it wasn't clear which of these factors might have contributed to the evolution of the shells we see today. Portugal and his colleagues decided to find out what influences have shaped the eggs of modern British birds (p. 3326).

However, instead of heading out to reserves and wildernesses, the team converged on the egg collection of the UK's Natural History Museum (Tring), where they could access samples of eggshell from over 150 different species – ranging from the common starling (*Sturnus vulgaris*) to the capercaillie (*Tetrao urogallus*) – to find out how well they lose water vapour. Fastening a segment of each eggshell across the mouth of a tiny test tube containing a 200 μl droplet of water, the team measured how much water was lost each day by evaporation. The team explains that under natural conditions, the water loss rates are likely to be similar across species; however, they say, 'Only under standard laboratory conditions will differences due to structural adaptations of the avian eggshell…become apparent.' Then the team compiled a comprehensive database of parental lifestyle factors – including breeding range, nest type, diet, habitat and whether the parent returned to the nest with wet feathers – before building a family tree incorporating each of the species to investigate which lifestyle factors most affected the rate of water loss.

However, when the analysis was complete, the team was surprised to see that factors that they had thought would influence eggshell water loss rates did not. 'We did not detect an effect of clutch size and developmental mode as significant main predictor variables of gas transfer', they say. Instead, eggs that are incubated by parents that routinely return to the nest with wet plumage have higher water loss rates than eggs incubated by dry parents, to compensate for the humid conditions and maintain optimal water loss rates over the course of incubation. Likewise, eggs that are incubated in confined and humid conditions, such as cup nests and burrows, tend to have higher water conductance than eggs reared in more open environments on the ground and in crevices.

'Taken together, these comparative data imply that species-specific levels of gas exchange across avian eggshells are variable and evolve in response to ecological and physical variation resulting from parental and nesting behaviours', the team concludes.

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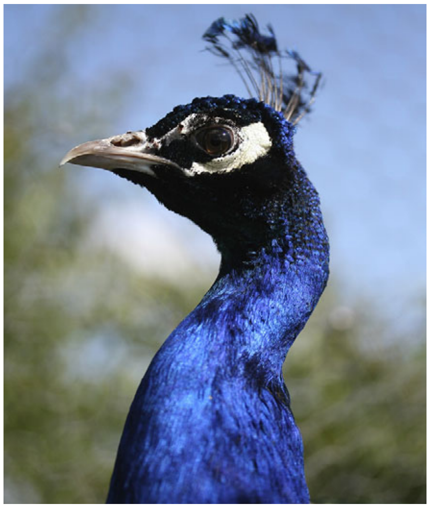
Portugal, S. J., Maurer, G., Thomas, G., Hauber, M. E., Grim, T. and Cassey, P. (2014). Nesting behaviour influences species-specific gas exchange across avian eggshells. *J. Exp. Biol.* **217**, 3326-3332.

Kathryn Knight

Take-off with train is no drag for peacocks



Head of a peacock (*Pavo cristatus*). Photo credit: G. Askew.

Peacocks are in a league of their own when it comes to performing for the ladies, strutting about with their ostentatious fanned trains; what female could resist? Graham Askew from the University of Leeds, UK, explains that males from many branches of the animal kingdom advertise themselves with elaborate ornaments, but these extravagant displays come at a price. ‘It is thought that such sexual traits have a negative effect on an individual’s performance…but that more elaborate ornaments indicate superior genetic quality’, says Askew. In the case of the peacock, he suspected that the train could restrict their flight – possibly costing them their lives in the event of an impaired escape bid – yet no one had ever measured the impact of the peacock train on the bird’s ability to take-off. ‘Trying to measure the effect that it [the train] had on performance seemed like a worthwhile effort’, says Askew, who decided to investigate how much an escaping peacock is incapacitated by his feather burden (p. 3237).

Selecting five Indian peacocks with intact feather trains, Askew startled the birds into take-offs as he filmed them in 3D with two high-speed video cameras. He then relieved the birds of their elaborate plumage, mimicking the natural loss of their trains at the end of the breeding season, and filmed their now unencumbered take-offs. Analysing the birds’ trajectories over the first three wing beats, Askew calculated the position of each bird’s centre of mass, their wing motions and the movement of the train. Then, he calculated how the loss of the train had altered the birds’ take-off and was amazed to see that it had little impact on their escape performance. The amount of power used by the birds to accelerate and gain height over the first two wing beats was essentially the same ($\sim$200 W kg^{-1}), regardless of the presence or absence of the train.

‘Intuitively, you expect that the train would affect flight performance and so not finding a detectable effect was a bit surprising’, admits Askew. Puzzled, he investigated how much the train affected drag on the birds during take-off. Mounting a detached train in a wind tunnel and measuring the drag on the feathers, he found that the drag increased by 200%, doubling the amount of power that the birds have to produce. However, the power that the birds have to produce to overcome drag is only 0.1% of their total aerodynamic power. So, the impact of the train on their overall take-off performance is negligible, allowing birds with and without trains to invest the same amount of power in the ascent, rather than having to divert some of it to overcome the effects of drag on the extravagant feathers.

Having shown that the train does not affect the peacock’s take-off, Askew says, ‘These results do not necessarily mean there are no costs associated with possessing an ornate train’. He points out that there is a range of other aspects of peacock performance that the train could affect, such as flight stability, running and the shear cost of producing such an impressive ornament – the peacocks invest 3% of their basic daily metabolic budget in train growth – and he suspects that all of these factors could contribute to some extent to the price that proud peacocks pay to lure in the ladies.

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Askew, G. N. (2014). The elaborate plumage in peacocks is not such a drag. *J. Exp. Biol.* **217**, 3237-3241.

Kathryn Knight

Rats whisk whiskers to sense surroundings

Watching a rat's rapidly shivering whiskers, you might be forgiven for thinking that the animal was nervy, but Mitra Hartmann, from Northwestern University, USA, explains that far from expressing anxiety, the animals are deftly exploring their surroundings. 'Rats use their whiskers to navigate, to search for objects and explore them, to socialize with other rats, to sense fine textures and to catch prey', explains Hartmann. However, it wasn't clear how sweeping their whiskers back and forth (whisking) affected the extent of the surface probed by the animal's tactile hairs. 'We wanted to learn where around the rat's head the rat could feel with its whiskers and how this "sensing region" changes during whisking', explains Hartmann. Measuring the area covered by the whisking whiskers in a live rat is almost impossible, so Hartmann and her colleague Lucie Huet modified the digital-rat simulation that had been built previously in the Hartmann lab to include realistic whisking motions, to investigate how rats perceive the world through the curved hairs (p. 3365).

After incorporating the complex equations that describe the whiskers' quivering motions into the digital-rat algorithm and tracking the whiskers' motions, the duo found that the tips of the hairs lay on the surface of an imaginary sphere centred between the animal's eyes. 'This suggests a tight coordination between the rat's whisker and visual systems', says Hartmann. In addition, the arrangement allows them to sense close approaching objects from almost all directions. The team also found that the curvature of the rat's whiskers allowed the animals to search 40% more space than if the hairs were simply straight. And the way that the rats tipped and twisted their whiskers while whisking to and fro allowed them to probe regions of space that were not searched by other whiskers. 'These small amounts of "elevation" and "roll" during whisking [were] long thought to be insignificant motions', says Hartmann. She concludes by explaining that the rats appear to be able to tightly control how finely they investigate an object. 'The rat often moves its front and back whiskers differently on the same side of the face', says Hartmann, adding that this could potentially allow rats to cluster the hairs close together when they want to build a detailed understanding of an object or spread them wide to search a larger space.

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Huet, L. A. and Hartmann, M. J. Z. (2014). The search space of the rat during whisking behavior. *J. Exp. Biol.* **217**, 3365-3376.

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