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Inside JEB

GETTING A GRIP ON CLIMBING FROGS' MOVEMENTS

Anthony Herrell

When it comes to getting around, most frogs prefer to hop; after all, years of evolution have resulted in a body plan fine-tuned for jumping. However, some members of the frog family have moved into the treetops, where jumping becomes a precarious way to move around. Instead, they resort to walking along the narrow walkways, constantly having to stabilise themselves as they navigate amongst the branches. But, they are not the only animals in evolutionary history to make this jump to arboreal life, and Anthony Herrel, a researcher at the National Natural History Museum, France, wondered how similar they are to these other arboreal species (p. 3599).

Outlining the aims of his team's study, Herrel says: 'The two things we were interested in were the actual grip types used and the basic mechanics of arm movement during locomotion and to see how that differed between substrates.' To do this the team turned to the South American tiger-leg monkey tree frog, *Phyllomedusa azurae*, famed for its monkey-like prehensile ability to wrap its tiny hands around branches. 'We had five cameras set up around a Plexiglas tank and in the tank we had different substrates, which were essentially metal wires of different diameters [1, 4 and 40 mm], and then we could orientate the tank horizontally or at a 45 deg angle,' says Herrel.

Herrel recalls that, at first, the frogs were very wary of their new surroundings. However, after a few months the frogs became accustomed to the tank and eventually the team were able to coax them to walk along the narrow walkway by providing them with a leafy hide-out at the other end of the wire. Having finally enticed them to move, the team could then begin to analyse the frogs' movements. They found that on the narrowest 1 mm wires, the walking frogs preferred grasping the wire between their second and third finger or between their second and fourth finger. The former grip, which the team call D23, was also their grip of choice when the narrow wire was inclined. Herrel and his team think that the D23 grip both helps stabilise the frog and can provide the most traction of all three grips identified, which, of course, is extremely important on inclines. In contrast, the third grip, where fingers three and four wrap around the branch, uses less grip force and perhaps for this reason is favoured on the horizontal and wider wires.

Although the grip types varied depending on the diameter of the walkway, the team didn't find as many differences in the way that the frogs moved. On the whole, the frogs were understandably slower on the narrower wires, taking up to twice as long to traverse the 1 mm horizontal wire. On the narrower walkways, the frogs also maximised their stability by lowering their body close to the wires as well as maintaining a good triangle of support by grasping onto the gangway with two hands and one foot or *vice versa*.

Overall, the frogs seem well adapted to walking on thin branches but what really stood out to Herrel is how similar these adaptations are to those of other arboreal and climbing species, including the primates: 'It's suggesting that these substrates are really imposing strong biomechanical constraints and animals really have to conform to this because there's quite an evolutionary history between them [frogs and primates] and still they're doing very similar things.' As this ability to grasp has evolved several times in the frog lineage, Herrel and his colleagues hope to understand exactly what drives the evolution of prehensile ability.

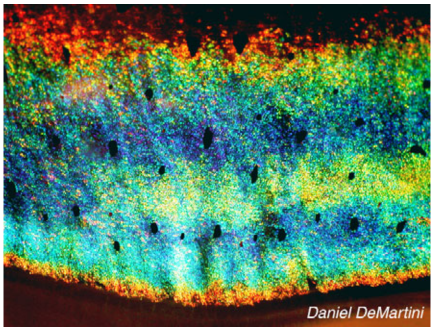
10.1242/jeb.094326

Herrel, A., Perrenoud, M., Decamps, T., Abdala, V., Manzano, A. and Pouydebat, E. (2013). The effect of substrate diameter and incline on locomotion in an arboreal frog. *J. Exp. Biol.* **216**, 3599-3605.

Nicola Stead

A FEMALE SQUID'S TRUE COLOURS

During his time in Daniel Morse's lab at the University of California Santa Barbara, USA, PhD student Daniel DeMartini has seen many *Doryteuthis opalescens* squid pass through the lab's doors. These squid provide DeMartini with a steady supply of the iridocyte cells that are responsible for the squid's shimmering opal-like markings. Iridocytes are found in many cephalopods, but what makes those of *D. opalescens* so special is their ability to adapt and produce a rainbow of colours from the same cell. Most iridocytes are found in patches across the squid's body but DeMartini recalls: 'We started to notice that some squid had bright iridescent rainbow stripes underneath their fins. Sometimes most of the squid in a batch would have them, sometimes none. After a while we started to realise the rainbow stripes were only seen in the females.' So after a few years of observing this, DeMartini decided to investigate this female-only trait further (p. 3733).



Daniel DeMartini

Upon the squids' arrival in the lab, DeMartini noted that on average, only half the females displayed colourful stripes, yet all were capable of producing them, as an hour after death all females had these vibrant markings adorning their bodies. When DeMartini examined the underlying tissue under a microscope he found it was full of iridocytes jam packed with layer upon layer of reflectins (the proteins responsible for reflecting the light as colour). The sheer number of iridocytes, each packed with a high number of reflectin layers, resulted in stripes that are six times brighter than other patches of iridocytes.

Sandwiched between the two colourful stripes, DeMartini also noticed a large bright white area whose appearance coincided with the emergence of the iridescent streaks. When he delved a little deeper he found that the underlying tissue was made up of leucophore cells. Like iridocytes, leucophores contain reflectin proteins, but instead of being arranged into layers, these light-reflecting proteins are packaged into rounded compartments throughout the cell. This alternative arrangement scatters light of all wavelengths, instead of reflecting just a single wavelength as colour, making the skin look white.

While leucophores are widespread in cephalopods, this is the first time switchable leucophores have been identified in *D. opalescens* squid. What's more, these leucophores are predominantly made up of reflectin subtypes that have only ever been found in adaptive iridocytes before. In iridocytes, these adaptive reflectins contract and change their refractive properties in response to the neurotransmitter acetylcholine, allowing them to fine-tune colour of the reflected light. But are they also adaptive in a leucophores? Sure enough, when DeMartini treated the female squid with acetylcholine the white region became brighter. 'This discovery reveals a fundamental relationship between the switchable leucophores and the tunable colour-producing iridocytes, suggesting they share a mechanism at the molecular level', says DeMartini.

So what is the purpose of these markings in females? In short, DeMartini doesn't know, but he points out that the white stripe looks remarkably similar to the white testes seen in male squid. He speculates that the iridescent stripes might give a 3D perspective to the white stripe: 'You could orient the iridocyte's reflection at some specific angle so it'll look brighter from certain positions, instead of white scattering which is always going to be uniformly bright in all directions.' As male squid are notoriously aggressive towards females, DeMartini suspects that these adaptable iridocytes and leucophores could help females mimic males to escape unwanted attention.

10.1242/jeb.094334

DeMartini, D. G., Ghoshal, A., Pandolfi, E., Weaver, A. T., Baum, M. and Morse, D. E. (2013). Dynamic biophotonics: female squid exhibit sexually dimorphic tunable leucophores and iridocytes. *J. Exp. Biol.* **216**, 3733-3741.

Nicola Stead

PRACTICE MAKES PERFECT WITH HELP FROM *FoxP2*

Qianqian Chen and Zachary Burkett

To attract a female's attention, a male songbird needs to woo her with his courtship song. However, male songbirds aren't born pre-programmed with this song and have to learn it by mimicking their kinsman's songs, in much the same way us humans learn to speak. Just like us, practice makes perfect, and male songbirds will often practise every morning. But how exactly does practising help? It's a question that interests Stephanie White, a neuroethologist from the University of California Los Angeles, USA: 'We're interested in how the brain controls behaviour and how behaviour affects the brain.' In humans, the ability to speak properly relies on two important transcription factors, *FOXP1* and *FOXP2*, which regulate the expression of other genes. It seems that it's the same in birds, as knocking down *FoxP2* levels prevents them from accurately copying their tutors' melodies. But is *FoxP2* involved after initial learning? From her previous work, White knew that expression of the *FoxP2* gene decreased when zebra finches practised their songs, but wondered whether this happened in other birds (p. 3682).

White recruited her graduate student, Qianqian Chen, onto the project and the pair decided to see what happened to *FoxP2* levels in Bengalese finches. White explains that these finches were originally bred from white-rumped munia for their colourful plumage: 'That freed up the male from needing to keep his song recognisable to the female in the wild, to evolving to her preference for more interesting songs.' Comparing them with zebra finches, which have predictable songs that follow a strict musical 'score', White says: 'Bengalese finches have beautiful phrases [songs] that in the middle can go one way or the other. Their song is less stereotyped and stuck in adulthood, so we wanted to know whether *FoxP2* variation would be more dramatic in them because they retain this greater flexibility in their song in adulthood.'

A week before the experiment began, Chen placed her male Bengalese finches into sound-attenuating chambers. On the day of the experiment she woke her birds up at 8 am and let half of them serenade the room as they practised their tunes. To prevent the other half from practising, Chen would keep a close eye on them, distracting them if they looked as if they were about to burst into song. At various time points: 1, 1.5 and 2 h, Chen would then collect brain samples to analyse for *FoxP2* expression.

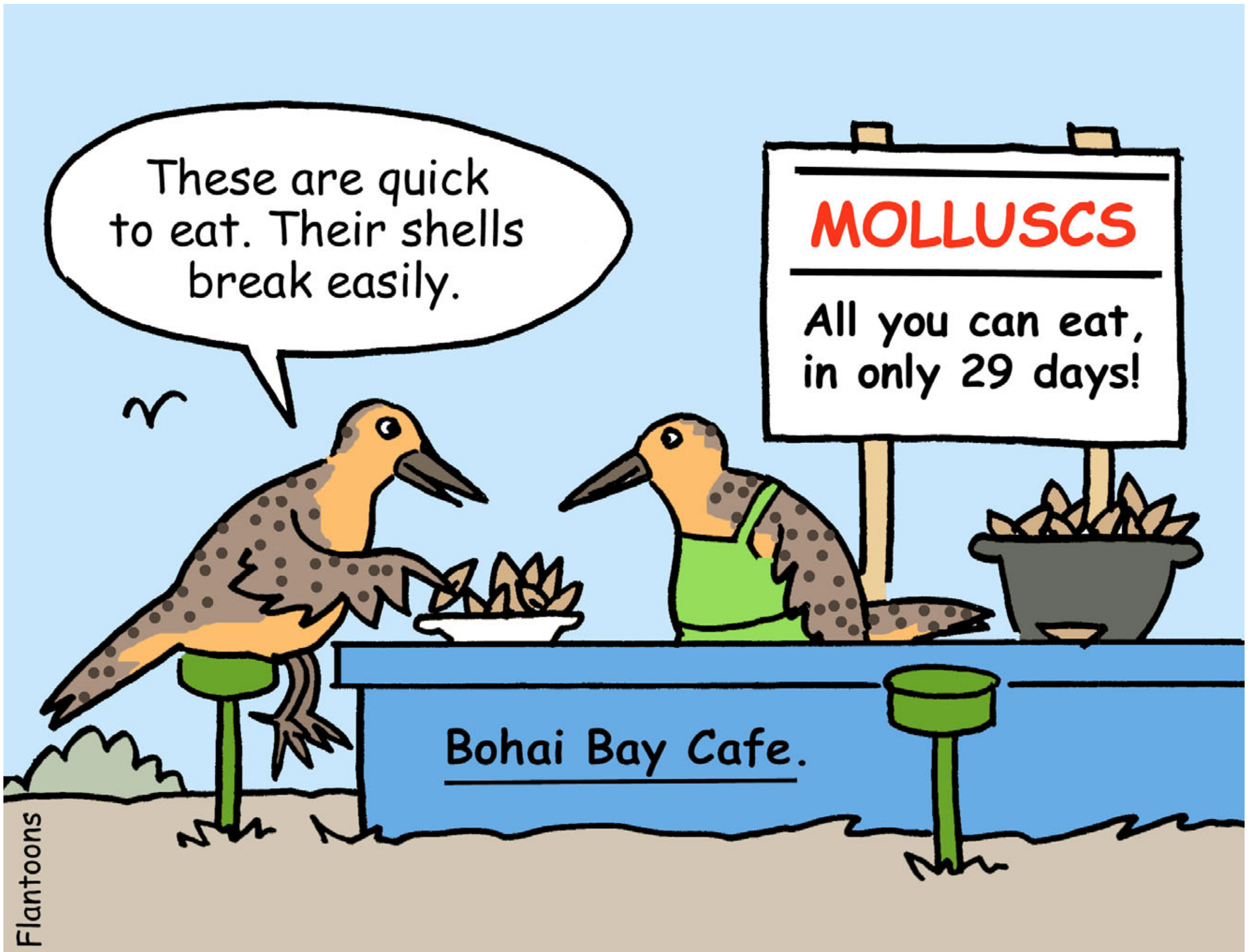
As with zebra finches, the team found that *FoxP2* levels in Area X (the brain region involved in song learning) did decline after 1.5 h, confirming that *FoxP2* plays an important reinforcing role in perfecting and maintaining courtship songs in birds. However, White recalls that this decline was: 'Surprisingly not more rapidly nor to any greater degree than in zebra finches. Which left us wondering, had we properly controlled for this bird's behaviour?' Sure enough, the Bengalese finches weren't as chirpy as their distant zebra finch relatives, and sang half as much during the entire trial. Unfortunately, because of circadian rhythms, the team couldn't extend the trial time, but by extrapolating from their data they think that *FoxP2* levels wouldn't go down any further or any faster than in zebra finches, despite the Bengalese finches' substantial musical repertoire. It's clear that *FoxP2* is a key player in this cycle of practice and performance and it's likely to be the same for us, as White explains: 'With these trial and error types of behaviour, you can't just pick them up once, you have to keep them up, and you're basically fine-turning your own levels of genes in the brain.'

10.1242/jeb.092817

Chen, Q., Heston, J. B., Burkett, Z. D. and White, S. A. (2013). Expression analysis of speech-related genes *FoxP1* and *FoxP2* and their relation to singing behvior in two songbird species. *J. Exp. Biol.* **216**, 3682-3692.

Nicola Stead

CRUSHABLE MOLLUSCS KEY TO BOHAI BAY'S POPULARITY



As they make their long Artic-bound journeys from their wintering grounds in the southern hemisphere, red knots make at least one short pit stop. They need to refuel fast, and if prey quality is low, they will usually increase the size of their gizzards so they can handle more food. However, if the menu is good, they'll reduce their gizzards instead, to save hauling excess weight around. Red knots travelling up from Australia and New Zealand usually stop over at Bohai Bay, in China and Theunis Piersma, from the University of Goningen, The Netherlands, wondered what fuelling opportunities were on offer at these mud flats. To find out he assembled a team (p. 3627).

By collecting the birds' droppings, the team found that red knots mainly eat the most available, *Potamocorbula*, molluscs. However, these tiny bivalves, with their low flesh to shell ratio, provide little energetic value – just 1.32 kJ g^{-1} dry shell mass, almost three times lower than the food from the red knot's summer homes – and usually they would ignore them. What's more, when the team analysed the birds' gizzards they found that they hadn't grown but had in fact shrunk by 17–48%. With such measly fare on offer, and small gizzards, the knots would, on average, have an intake rate of just 2.7 J s^{-1}, well below the 3.6 J s^{-1} rate required to fatten up during their brief 29 day layover.

So why do red knots keep stopping off at Bohai Bay? Piersma and his team decided to look at how quickly red knots could digest the shells. As the *Potamocorbula* molluscs are so small, they can be easily crushed and were processed at a speedy 3.93 mg s^{-1}. With such high rates, the overall energy intake rate increased to 5.1 J s^{-1} – more than enough to gain the 80 g of fat needed for their onward journeys. While *Potamocorbula* molluscs might not be a red knot's first choice, the crushability of these bivalves and their unique abundance at Bohai Bay offers knots the best of both worlds – the ability to stock up quickly and reduce gizzard size.

10.1242/jeb.094607

Yang, H.-Y., Chen, B., Ma, Z., Hua, N., van Gils, J. A., Zheng-Wang, Z. and Piersma, T. (2013). Economic design in a long-distance migrating molluscivore: how fast-fuelling red knots in Bohai Bay, China, get away with small gizzards. *J. Exp. Biol.* **216**, 3627-3636.

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