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INSIDE JEB

Orangutans use hand like soundbox to make alarm calls

Wild male Sumatran orangutan in the Ketambe research area, Indonesia. Photo credit: Adriano Lameira.

Deep in the rainforests of Sumatra and Borneo you might be surprised to hear a familiar sound: kissing. But when solitary orangutans make the noise it is no romantic gesture. 'Orangutans make these 'kiss-squeak' alarm calls if humans and dangerous animals come near', explains Bart de Boers from the Vrije Universiteit Brussel, Belgium. And when Madaleine Hardus from the Pongo Foundation and Adriano Lameira and Serge Wich from the University of Amsterdam, The Netherlands, first discovered the threatening sound, they noticed that some animals cupped their hands around their muzzles, noticeably lowering the pitch that they produced. Were the apes trying to sound bigger and more threatening than they really were? Chatting to Lameira, de Boer realised that he might be able to help the biologists find out whether the animals were really modifying their alarm calls to create a false impression. 'I said, "this sounds like a really interesting thing to model because then you will know for sure whether using the hand really helps to exaggerate size" ', recalls de Boer.

But first he had to build two mathematical models – one to understand the sound production mechanism and the other to simulate the frequency signature – to learn more about how the apes deepen the alarm sound. Describing the first model, de Boer explains that he simulates the lips as a thin tube opening through a disc that represents the face. Then, he places a second disc a short distance above the face disc, to simulate the ape's hand. Deriving a set of equations that described how sound travels through the lips and then bounces back and forth in the cavity between the orangutan's face and hand, de Boer realised that by channelling the sound through the hand and face cavity – like the sound box of an instrument – the ape was effectively lengthening the pipe structure to lower the sound's pitch and make it deeper.

But simply lowering the pitch of a note is not enough to make it sound as if it is produced by a larger structure. The overtones that accompany the fundamental frequency and make the sound richer have to be lower too. If the orangutans were really going to pull off the feat and pass themselves off as being larger, they would have to generate more low overtones in their kiss-squeak calls. To test this, de Boer built the second more sophisticated model to calculate the frequency spectrum of calls and was very impressed to see that the lower overtones were amplified in the simulation when the hand was in place.

But how well did de Boer's calculations compare with Lameira and Hardus's hard-won recordings? 'The problem is that the orangutan is not sitting still on a branch making its noises' chuckles de Boer. 'There are cicadas singing in the background, rustling leaves – all kinds of horrible stuff going on', he adds. However, after painstakingly removing the background cacophony, de Boer was amazed to see how well the simulations agreed with the real thing. Not only did the mouth-cupping orangutans sound deeper, but they had managed to amplify the deeper overtones that make them sound larger.

And de Boer is excited because this might be the first hint that an animal can learn to modify sound, which is an essential tool for language acquisition. 'Orangutans may be aware that they can influence their call and it changes the reaction of the predator, and this a simple form of learning, which is a very important first step in language', he says.

10.1242/jeb.121624

de Boer, B., Wich, S. A., Hardus, M. E. and Lameira, A. R. (2015). Acoustic models of orangutan hand-assisted alarm calls. *J. Exp. Biol.* **218**, 907-914.

Kathryn Knight

New model captures essence of walking

Motion capture cameras record participant walking on treadmill. Photo credit: Karl Zelik.

Karl Zelik wants to help people. He wants to build better prosthetic limbs for amputees and help surgeons improve the care offered to children with cerebral palsy. But before he can realise this mission, Zelik needs to understand the way that people move naturally and the clever energy-saving mechanisms that our bodies use. To do this, he analyses the movement of our limbs and the forces they generate to calculate how various muscles and joints perform work during locomotion (inverse dynamics). However, Zelik noticed recently that the sums weren't adding up. When reanalysing data collected earlier on walkers in Art Kuo's lab at the University of Michigan, USA, Zelik realised that the inverse dynamics approach couldn't account for 25% of the energy changes during a stride cycle. Zelik adds, 'We could not identify which muscles, tendons, joints or segments were responsible for generating this movement'. Deciding that the conventional inverse dynamics approach was too simplistic, Zelik and his colleagues Kota Takahashi and Greg Sawicki, both from North Carolina State University and the University of North Carolina at Chapel Hill, USA, decided to build a new, more realistic model of our body movements to see if the trio could account for the missing energy and to find out where it was coming from.

Recruiting ten healthy students to walk at speeds ranging from a leisurely saunter to a brisk march on a treadmill, Zelik measured the forces that each individual exerted on the ground as their feet pushed down while using a sophisticated 3D motion capture system to record each movement in fine

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detail. Then, based on the force measurements, Zelik, Takahashi and Sawicki calculated the total kinetic and potential energy of each walker's body over individual stride cycles. Finally, having analysed the walkers' limb and body motions from the 3D reconstructions of their movements, the trio built a series of models with increasing complexity – ranging from the simplest, which accounted only for the rotational movements about each limb joint (three degrees of freedom model); to a model that included the contribution of the work done by the swing limbs and the muscles and tendons in the foot (three degrees of freedom plus foot); and the most complex model, which incorporated sliding motions and tissue compression in the hip, knee, ankle and foot (six degrees of freedom) – to calculate how much work each joint and body segment contributed to their movements.

Comparing the total energy values that had been calculated for the whole body with the mechanical work calculated from each of the models based on the body segment motions, Zelik and his colleagues were delighted to see how well their most sophisticated model agreed with the whole body energy calculations. 'It all added up properly,' says Zelik, adding, 'that positive mechanical work performed by the body's joints and segments actually summed up to explain the change in energy on and about the body's center-of-mass was a surprise'. And when the trio analysed the individual contributions of different body segments and joints to walking, they realised that the hip muscles make a more significant contribution than had been previously recognised.

'This study is the first to demonstrate that we could directly link joint- and segment-level work sources with the observed energy changes of the body during locomotion', says Zelik, who is now keen to learn more about how changes in our bodies, such as weight gain, affect walking. He adds, 'This new approach has implications for assistive technology design, musculoskeletal walking simulations and potentially clinical treatment'.

10.1242/jeb.121590

Zelik, K. E., Takahashi, K. Z. and Sawicki, G. S. (2015). Six degree-of-freedom analysis of hip, knee, ankle and foot provides updated understanding of biomechanical work during human walking. *J. Exp. Biol.* **218**, 876-886.

Kathryn Knight

Xenopus males lose characteristics through different strategies

Female (left) and male *Xenopus borealis*. Photo credit: Elizabeth Leininger.

Some males really go to town when putting on a show for the ladies. Male peacocks flounce around with elaborate feather trains while male dear strut their stuff with impressive antler racks. However, other males prefer to serenade the ladies. 'Most frog songs fall into this rubric with males exhibiting more showy and elaborate forms than females,' says Elizabeth Leininger from St. Mary's College of Maryland, USA. However, she adds that showy male traits can be lost as well as gained, 'either by females assuming the male character or via the loss of masculine traits,' she says. While most *Xenopus* males regale the ladies with rapid and elaborate calls, the males of two distantly related species – *X. boumbaensis* and *X. borealis* – have relinquished the more complex details of their mating calls. Intrigued by mechanisms that led to the loss of male characteristics and knowing that the larynx probably plays a key role in forming those differences, Leininger and Darcy Kelley from Columbia University, USA, decided to compare the male and female larynges of *X. boumbaensis* and *X. borealis* that had lost the male characteristics in their calls.

Measuring the relative sizes of the frogs' larynges, the duo could see that the male *X. boumbaensis*' larynges were ten times the size of the females' because they had more muscle fibres, the muscle cells were colossal and they had a larger cartilage box. Meanwhile, male *X. borealis*' larynges were only five times larger than their females'. Next, Kelley and Leininger began comparing the muscle fibre types in the frogs' larynges – there are two types of muscle fibre, ones that contract and relax rapidly (fast-twitch) and slower contracting (slow-twitch) fibres – to look for differences between the males and females. Teaming up with Ken Kitayama, Kelley and Leininger could see that muscle fibre distribution in the larynges of male *X. borealis* was similar to that of the female muscle fibres; both had a mixture of fast- and slow-twitch fibres, which would naturally restrict the laryngeal muscle contraction and prevent *X. borealis* males from producing the rapid and complex courtship calls produced by other *Xenopus* males. However, when they investigated the larynges of both sexes of *X. boumbaensis*, they found that the males' larynges were nothing like the females'; the male laryngeal muscle fibres are exclusively fast twitch and looked more like the larynges of other *Xenopus* males.

Next, the duo focused on nerve stimulation of the laryngeal muscles to find out how the muscle physiology varied between the males and females of each species. The intensity of most *Xenopus* males' calls increases toward the end of a trill and Kelley and Leininger explain that this occurs when closely timed electrical stimuli reinforce the earlier stimuli (known as potentiation) to trigger progressively stronger contractions and louder calls. When the team measured the characteristics of the laryngeal muscle contraction in response to nerve impulses, they found potentiation in male *X. boumbaensis*, but less in the females. However, the male *X. borealis*' response to electrical stimulation was more like that of the females, lacking the potentiation that increases the volume of the mating call in other *Xenopus* males.

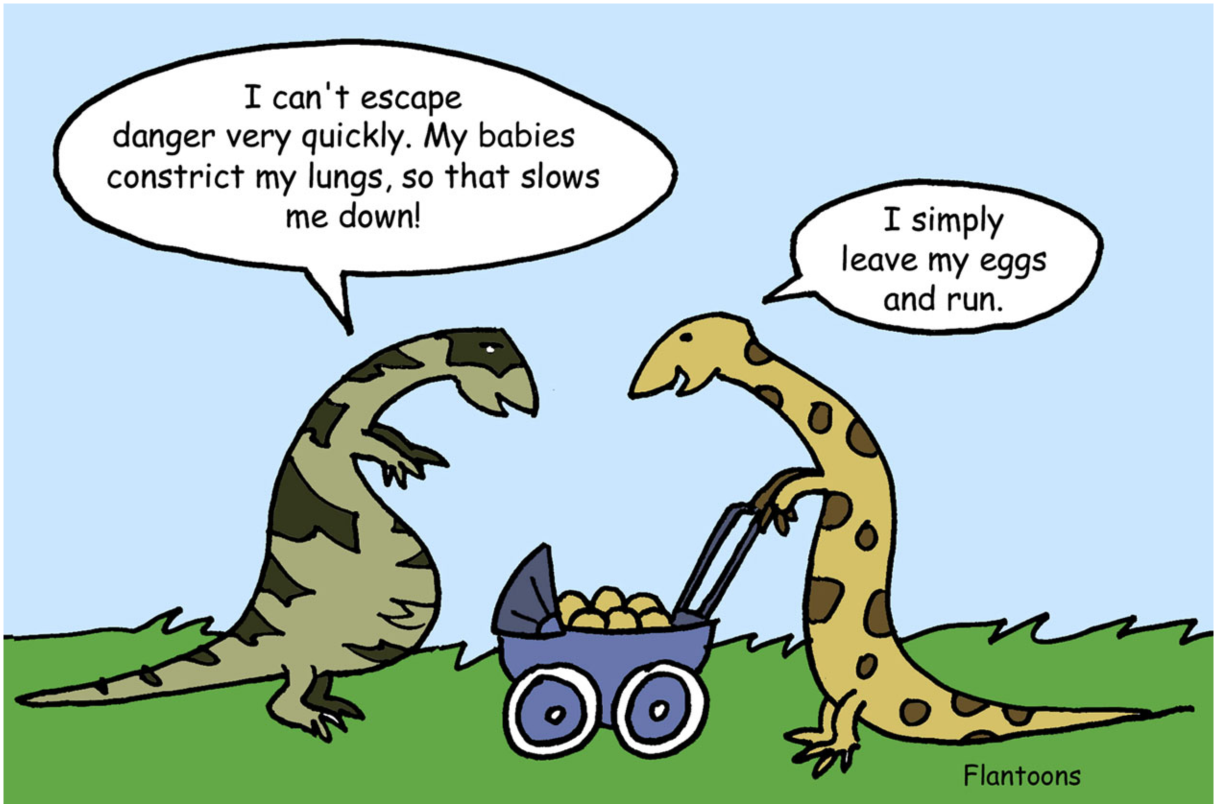
So, the loss of male features in the *X. borealis* mating call is associated with the feminisation of their larynges. And, although the *X. boumbaensis* larynx retains many of the physical characteristics associated with other *Xenopus* males, Leininger explains that they do not have the appropriate patterns of input from the brain to produce the elaborate calls used by the majority of *Xenopus* to woo the ladies.

10.1242/jeb.121632

Leininger, E. C., Kitayama, K. and Kelley, D. B. (2015). Species-specific loss of sexual dimorphism in vocal effectors accompanies vocal simplification in African clawed frogs (*Xenopus*). *J. Exp. Biol.* **218**, 849-857.

Kathryn Knight

Pregnancy compresses skink mums' lungs



Compared with most pregnant mums, skinks have it easy. They invest much of the energy needed to rear their young to full term when producing the egg yolk that will nourish the foetuses during development. But that doesn't mean that skink mums get off scot-free: the additional burden of carrying a litter impairs their mobility and messes with their metabolism. 'The physiological mechanisms that underpin these gestational costs are poorly understood,' says Suzanne Munns from James Cook University, Australia. Intrigued by the challenges faced by blotched blue-tongued skink mums-to-be, Munns and a team of scientists from the University of Tasmania, Australia, including Ashley Edwards, Stewart Nicol and Peter Frappell, measured the breathing patterns, metabolic rates, lung volumes and gas diffusion across the lungs of pregnant and non-pregnant female skinks to find out how they cope with pregnancy.

Exercising the animals, the team found that the non-pregnant females were able to rise to the challenge and increase their metabolism over 300% while breathing much deeper and faster. However, the females that were in the late stages of pregnancy were unable to alter their breathing patterns or increase their metabolism as they exercised. And when the team measured lung volume in the pregnant mums, the lung volume increased over the duration of the pregnancy, but increased even more after giving birth, suggesting that the lungs were compressed by their litter. However, gas diffusion across the lung was not affected by pregnancy. Explaining that skinks do not use the entire surface of the lung for gas exchange, the team suspects that the portion of the lung that acts as a bellows moving air around the lung is compressed during pregnancy. They say, 'the lack of ability to respond to increased respiratory drive may be one of the mechanisms that underpin the locomotor impairments measured in pregnant lizards'.

10.1242/jeb.121657

Munns, S. L., Edwards, A., Nicol, S. and Frappell, P. B. (2015). Pregnancy limits lung function during exercise and depresses metabolic rate in the skink *Tiliqua nigrolutea*. *J. Exp. Biol.* **218**, 931-939.

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