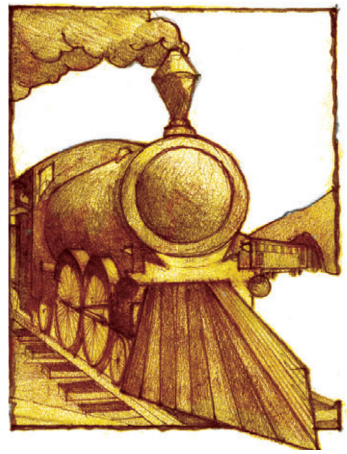
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

REPRODUCTIVE BURDEN



ARE THE KIDS WEIGHING YOU DOWN?

As a new father of twins, I have an all too acute and intimate familiarity with some of the costs associated with reproduction (never did I imagine yearning for 4 h of sleep, for example). However, compared with my wife, I've had it, and still have it, relatively easy. After all, I didn't spend over 9 months gestating, not to mention giving birth to, two infants. Reproductive costs for all kinds of animals have been studied widely among behavioral ecologists, particularly in relation to the burden carried (literally) by pregnant females. For example, a number of studies of squamate reptiles have shown reduced sprint speed and/or endurance capacity in pregnant animals. What has remained unclear in many of these studies is the extent to which the performance decrement is due to physical or physiological causes. Is it simply the added weight of the carried offspring that underlies reduced locomotor capacity in pregnant females or might there be physiological changes that are to blame?

To help tease apart the importance of physical *versus* physiological bases of so-called reproductive burdens, Peter Zani, at Lafayette College, and colleagues Ryan Neuhaus, Trevor Jones and Jonathan Milgrom studied locomotor endurance in hundreds of side-blotched lizards (*Uta stansburiana*) over the course of their natural breeding season for 2 years. Zani and colleagues worked on populations in eastern Oregon, USA, collecting lizards in late spring and early summer of 2005 and 2006. After collecting the lizards in the morning and taking them to a field lab, the team carried out endurance trials on a motorized treadmill. Having determined the time and distance traveled before exhaustion, the team sexed the lizards and categorized females further as non-reproductive, with enlarged follicles, yolked eggs or shelled eggs, or postreproductive. All animals were marked and released within 48 h except females with shelled eggs, which were kept until after ovipositing, when they again underwent a treadmill trial to assess how rapidly they re-gained their endurance.

In general, females exhibited lower endurance capacity than males, and as their reproductive cycle progressed, endurance progressively diminished, such that females carrying shelled eggs ran distances approximately 15–20% shorter than their non-reproductive counterparts. Further, data showed that following oviposition, animals recovered their non-reproductive endurance levels in a very short time – within a 12 h window – leading Zani and colleagues to suggest that the burden carried by females of this species is largely a physical one since physiological effects, such as altered blood-hormone levels, would probably last for substantially longer time periods. More manipulative experiments will be required to clearly elucidate the relative importance of physiology and physical load when evaluating the reproductive burden faced by female lizards. But what this study makes clear is that a burden with a strong physical component is indeed faced in this species and the nature of the burden remains quite consistent from year to year.

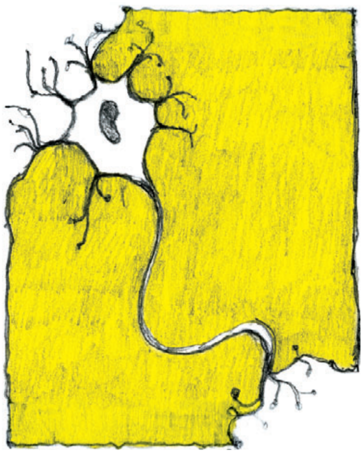
After explaining this work to my wife, she acknowledged a degree of empathy for these female lizards, as she too suffered a serious physical burden as her own pregnancy progressed. However, this empathy was short lived once she found out the lucky lizards recovered from their deficits in a mere 12 h…

10.1242/jeb.011668

Zani, P., Neuhaus, R., Jones, T. and Milgrom, J. (2008). Effects of reproductive burden on endurance performance in side-blotched lizards (*Uta Stansburiana*). *J. Herpetol.* **42**, 76-81.

Gary B. Gillis
Mount Holyoke College
ggillis@mtholyoke.edu

COMMUNICATION



TALK TO THE ANIMALS

Communication amongst members of a species is a crucial aspect of social behaviour and is important for survival. Our ability to discriminate our own species' vocal sounds from other sounds and to distinguish between the vocalizations from someone we know and someone we do not has been described down to the specific brain regions responsible. It is recognized that humans have greater vocal range and linguistic capabilities than other non-human primates and our specialized ability to communicate may at first seem to set us apart from other species. New evidence suggests, however, that this may be a hasty conclusion. A recent study by Christopher Petkov and colleagues, from the Max Planck Institute in Tubingen, Germany and from the University of Manchester, UK, published in *Nature Neuroscience* in March, 2008, has identified a specific 'voice' region in the brains of a species of Old World monkey, the macaque.

Functional magnetic resonance imaging (fMRI) studies in humans have identified a brain region that is sensitive to human voices and speech. It is unclear, however, whether this is a function of the linguistic processing of speech itself (because we communicate with words) or whether voice regions have been evolutionarily conserved in other primates. Behavioural evidence shows that in many primates, their attention is drawn to the sound of their own species' vocalizations, but no one had experimentally identified a specific auditory region dedicated to the recognition of vocalizations from their own species.

By using fMRI to measure brain activity while macaques listened to a variety of sounds, this study sheds light on a region of the macaque brain that shares a functional relationship with the human voice region, supporting the idea that different primate species possess brain regions adapted to recognizing communication signals from their own species.

First, the researchers investigated which cortical regions became active when the macaques listened to different sounds. Using four different categories of sound – specific macaque vocalizations, other animals' vocalizations, natural sounds and an acoustic control (consisting of sounds of a similar pitch and duration to those from the other categories) – they identified a discrete brain region that reacted robustly in response to the macaque sounds but exhibited only a modest response to the other three categories of signals. This region was identified as a hierarchically high-level processing region in the anterior of the auditory cortex. Importantly, other regions of the auditory cortex were also activated, but this study was particularly interested in identifying a region solely adept at recognizing the sounds of its own species. Further experiments determined that the anterior region, and only this region, became activated in macaques listening to the vocal sounds of familiar macaques and that the sounds of unfamiliar individuals did not elicit a similar activation pattern. This is evidence that the anterior region is sensitive to the identity of the 'speaker'. Altogether, this study highlights the existence of a voice region in macaque monkeys that has functional properties comparable to those described in humans.

Social animals depend on communication with their own species for survival. If the voice region is not unique to us, evolution likely influenced different primate species and other animal species to evolve similarly. With this in mind, perhaps we should be careful what we say around our pets: who knows what they might be picking up.

10.1242/jeb.011676

Petkov, C. I., Kayser, C., Steudel, T., Whittingstall, K., Augath, M. and Logothetis, N. K. (2008). A voice region in the monkey brain. *Nat. Neurosci.* **11**, 367-374.

Sarah Hewitt
University of Calgary
sahewitt@ucalgary.ca

SONG GENERATOR

SHE CAN (ALMOST) SING LIKE HIM

Guys have a way of laying on the charm when a relationship starts. The man does his best to show only his finest attributes while wooing his girl. This is quite a common theme in virtually all animal courtship. Even the male fruit fly taps into all the magnetism he can muster in luring a mate. One of the many rituals that the male fruit fly performs during the courtship dance is the vibration of his wings. By quivering his wings just so, he sings to entice his chosen mate. Only the male serenades while only the female consents to the 'come on'.

What is surprising is that both male and female flies appear to be hardwired with the ability to sing. This ability may be controlled like an 'on–off' switch in appropriate neurons. Females may contain the necessary song neurons but they remain dormant without the trigger necessary to turn them 'on'.

In the April 18, 2008 issue of *Cell*, Gero Miesenbock from the University of Oxford and Yale University School of Medicine, and his postdoctoral fellow, J. Dylan Clyne, address the question, 'What accounts for the uniquely male propensity for producing song?' Maybe male wiring is slightly different from the females'.

Knowing that certain genes are expressed differently between the sexes, the team began by targeting *fruitless* (*fru*), a regulatory gene in the nervous system that is expressed differently in males (Fru^{M}) and females (Fru^{F}). By labeling *fru* neurons with green fluorescent protein (GFP), the team was able to illuminate a key subset of singing neurons and directly compare male and female brain circuitry. Lighting up Fru to display the song circuit allowed Clyne and Miesenbock to visualize the way the neurons are wired to one another in the brain and to find that male and female fly brains contain a similar network of the song-producing neurons, termed the song generator.

If both sexes contain the basic anatomical equipment to produce song, what allows the male to sing while the female remains silent? To answer this question, Clyne and Miesenbock subtly modified the *fru* gene so that expression of the gene could be artificially turned on in females' neurons with a flash of light, and listened for song. The team filmed flies and showed that by switching on selected neurons they were able to trigger singing in females. However, the flash of light needed to be brighter than that required to activate the males' song generator. And when the duo listened to the songs of males and females they found that the females sang out of tune. It appears that, in terms of courtship song, males remain the showy experts.

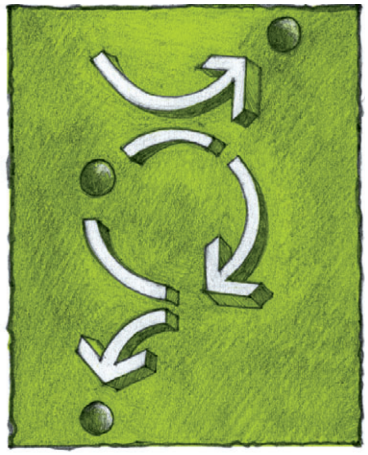
The authors suggest that differences in the males' and females' abilities to vibrate their wings and sing may be explained by one of two scenarios. Either, (1) very subtle differences in brain anatomy ensure male behavior remains exclusively male (and female behavior remains exclusively female), or (2) the critical on–off switch in key subsets of neurons are set in opposite configurations within the two sexes (male song-generator neurons are switched 'on' by Fru^M, while the generator is turned 'off' in females). Still, it is remarkable that a principally male behavior can even be elicited from females. By simply modifying a single gene's expression within a subset of neurons, scientists are able to evoke an unnatural behavior. Deeper insight into the behavioral differences between the sexes (in flies) will be revealed when the downstream genes that are regulated by Fru are uncovered.

10.1242/jeb.021105

Clyne, J. D. and Miesenbock, G. (2008). Sex-specific control and tuning of the pattern generator for courtship song in *Drosophila*. *Cell* **133**, 210-212.

Katherine M. Parisky
Brandeis University
kparisky@brandeis.edu

EXERCISE CAPACITY



GETTING TO THE HEART OF EXERCISE

Cardiovascular disease is unlikely to be a concern for the many amazing athletes at this year's summer Olympics. This is because exercise capacity is related to heart function, and a strong healthy heart is important for pumping blood around the body during exercise. Being a good athlete and having a strong heart depends a lot on genetics, which can contribute half of the variation seen in human exercise capacity. Some other animal species have also evolved the ability to perform astounding feats of athleticism, often with very little training. However, in humans or animals from the wild it can be very difficult to separate the genetic basis for exercise capacity, heart function and cardiovascular disease from the effects of training and environment. Anja Bye and colleagues from the Norwegian University of Science and Technology therefore decided to examine this topic in rats artificially selected for running endurance.

Artificial selection is a way of mimicking natural selection in the lab. Every generation, individual rats with the best and worst running endurance were selected and bred together, generating lines with high and low capacities for running long distances. The authors knew that hearts from the athletic line could pump more blood than those from the unathletic line, because their hearts were bigger and their heart muscle fibres could contract more. They also knew that the low-capacity runners had risk factors for cardiovascular disease, like high blood pressure and elevated lipid levels in the blood. To better understand the underlying molecular basis for these differences, Bye and colleagues compared gene expression in the hearts of these high- and low-capacity rat lines.

By measuring the expression of 28,000 genes with gene chips, small microarray slides that quantify the expression of many different genes at once, the authors found that 1540 genes were differentially expressed between high- and low-capacity runners. Many genes were expressed at higher levels in the athletic line, which could explain how their hearts perform better than those of the unathletic line. Contraction and calcium signalling genes were more highly expressed, potentially explaining how their heart muscle fibres are better at contracting. Expression of fat metabolism genes was also enhanced in the high-capacity line, allowing lipids to fuel the high metabolism of their hearts for prolonged periods. In contrast, genes important for carbohydrate metabolism and transport were more highly expressed in the unathletic line, similar to what happens in the hearts of humans with heart disease. Expression of genes involved in cell stress and pathological growth signalling was also higher in the low-capacity line, possibly because the hearts of low-capacity runners aren't getting enough oxygen but are constantly pumping blood at a higher pressure.

The results of this study suggest that there is a switch in gene expression between athletes and couch potatoes, from a pattern supporting lipid metabolism and heart muscle contractility to one that indicates an unhealthy heart. The authors have shown that many complex molecular changes contribute to the evolution of athleticism, and have generated novel hypotheses about the links between exercise capacity and heart disease. Although many unanswered questions remain, Bye and colleagues have brought us one step closer to understanding just how much heart it takes to get to the podium!

10.1242/jeb.011650

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Graham R. Scott
University of British Columbia
scott@zoology.ubc.ca

SUCKLING PHEROMONE

WILD AND DOMESTIC RABBITS DIFFERENTIALLY RESPOND TO MAMMARY PHEROMONE

Born naked and blind, new baby rabbits are completely dependent on their mothers. Unfortunately rabbits are rather relaxed mothers, nursing their young only briefly once a day, so it is essential that the pups make the most of mum's brief visits. They are stimulated to suckle immediately by a pheromone, 2-methylbut-2-enal, released with their mother's milk. Fortunately, rabbit pups don't have to rely on their laid-back mothers for long. Developing in a matter of weeks, domesticated rabbit pups are soon able to forage for themselves, and wild youngsters grow up even faster. Knowing this, Gerard Coureaud and his colleagues in France and Germany wondered whether rabbit pups' sensitivity to maternal chemosignals is restricted to the period when they are dependent on milk and their eyes are closed, and whether the wild and domesticated pups show the same sensitivity to suckling pheromone as they begin to rely less on milk.

First the team tested the responsiveness of youngsters to the mammary pheromone during the course of lactation. Purchasing the mammary pheromone, they allowed leverets to sniff the compound for a 10 s period each day of lactation and once after weaning. The team tested a colossal number of domesticated and wild pups, never testing the same pup twice, as they measured the youngsters' sensitivity to the pheromone from birth to weaning.

By observing how often the pups latched on to the glass stick on which the pheromone was presented and started sucking, the researchers were able to classify the domesticated youngsters' responses to the pheromone. They found that while pup responsiveness was highest in the first few days of lactation, with 93% of all tested pups responding to the pheromone by suckling, it then began decreasing from week to week, reducing to about 50% in the third week, and vanished completely after weaning.

Next the team compared the pheromone responses of wild rabbits with those of their domesticated cousins and found that the wild pups responded just as strongly to the mammary pheromone at the beginning of lactation as the domesticated rabbits. However, the wild pups' sensitivity began decreasing during the second week of lactation and there was virtually no response to the pheromone by the final week of lactation. The wild pups ceased chemical communication with their mums about 1 week earlier than the domestic pups.

Coureaud and his colleagues suspect that domesticated rabbits have been artificially selected to rely on nursing longer than wild rabbits and therefore experience decelerated ontogeny. The fact that wild leverets are more motivated to search for solid food early in their development severs the pheromonal bond between pups and their mums much sooner in the wild than in the hutch.

Coureaud's findings nicely show that the mammary pheromone of the genus *Oryctolagus* is a good example for efficient chemical communication between a mother and her young to induce suckling behaviour in leverets that are dependent on milk.

10.1242/jeb.011635

Coureaud, G., Rödel, H., Kurz, C. and Schaal, B. (2008). The responsiveness of young rabbits to the mammary pheromone: developmental course in domestic and wild pups. *Chemoecology* **18**, 53-59.

Teresa Valencak
Veterinary University Vienna
teresa.valencak@vu-wien.ac.at

POSTPRANDIAL THERMOPHILY

IS POSTPRANDIAL THERMOPHILY AN EXPERIMENTAL ARTIFACT?

Ectothermic animals are generally thought to possess a species-specific preferred body temperature, which they protect behaviourally. For example, after feeding, many ectothermic animals appear to choose a higher temperature, termed postprandial thermophily, which may enable the animal to digest its meal at a higher rate. However, postprandial thermophily is generally investigated in laboratory thermal gradients. The open question is whether an animal's behaviour in this simplified setting is the same as observed in the wild? Does postprandial thermophily really exist? Some evidence suggests that it may not. For example, garter snakes maintain high constant temperatures in the field, but select relatively low temperatures in a thermal gradient, whereas other reptiles select higher temperatures in laboratory gradients than they do in the wild. Michael Wall and Rick Shine from University of Sydney, Australia, set out to investigate postprandial thermophily in an Australian pygopodid lizard (*Lialis burtonis* Gray) to test whether there was a discrepancy between field and laboratory measurements of postprandial thermophily.

To test the lizards' preferences when fed and unfed, the team designed three settings with different thermal patterns. First they constructed a field enclosure with a natural leaf litter layer that the lizards could burrow into, mimicking their natural habitat. The leaf litter provided good insulation, resulting in a temperature gradient ranging from 41.6°C at the surface to 24.8°C at the bottom of the leaf litter so that the team could measure the lizards' body temperatures after the reptiles had burrowed to a comfortable temperature. The authors also constructed a traditional temperature gradient in the lab with six visually separated sandy lanes. A combination of heating strips and cooling water created a linear temperature gradient at the sand surface with one end at 15°C and the other at 43°C. Placing fed and unfed animals at the warm end of the gradient, each in individual lanes, the team allowed the animals to run freely within the temperature gradient and measured their body temperatures for 3 days. But what if the fed lizards simply stayed put after feeding? The team had no way of knowing whether the reptile's immobility was due to postprandial thermophily or the animals simply settling down in any old spot to digest dinner. This time the authors adjusted the gradient so that the temperature ranged between 26.5°C and 50°C before placing fed and unfed animals in the cool end and waiting to see whether the reptiles moved. If the fed lizards moved to a warmer place than unfed lizards, then they were demonstrating postprandial thermophily. But if they simply settled down to digest their meal at the cold end of the gradient, then they would be cooler than the unfed animals; 'reverse' postprandial thermophily.

The outcome was in fact all three possibilities! In the field experiment there were no differences between the fed and unfed lizards' mean body temperatures, i.e. no postprandial thermophily. However, in the first gradient experiment the fed lizards had higher body temperatures than the unfed lizards; classic postprandial thermophily. Finally, the second gradient experiment showed that the fed lizards were cooler than the unfed lizards – there was reversed postprandial thermophily.

Michael Wall and Rick Shine have elegantly shown that thermal gradient data may be misleading, or even plain wrong, compared with free-ranging animals' thermal ecology. As most postprandial thermophily research has been carried out in temperature gradients in the lab, the team suspects that postprandial thermophily could be less widespread than previously thought. Researchers should, therefore, take care in future experiments to provide animals with microhabitats that better approximate their natural environment to get more physiologically relevant readings.

10.1242/jeb.011643

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Johnnie B. Andersen
University of Aarhus
johnnie.andersen@ki.au.dk