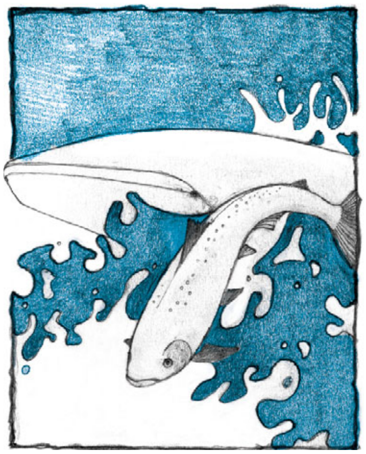
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

GILL DEVELOPMENT



ION REGULATION DRIVES GILL DEVELOPMENT

During the embryo and early larval stages, fish are small enough to sustain metabolism by simply diffusing O_2 across their skin. However, as the fish grows this becomes inefficient and a special structure with a larger surface area for extracting O_2 is required. At this crucial moment, fish begin to develop gills, the quintessential characteristic we associate with fish and their ability to 'breathe' in watery habitats. However, gills have many more functions. Adult fish also use gills to dispose of metabolic carbon dioxide and for pH and water balance, which is done largely *via* ion regulation pathways. This is important because the ion concentrations of a fish's body fluids are very different from those of the water in which it lives, allowing essential ions to leak either into or out of the fish's body to create ionic imbalances that disrupt crucial physiological processes. Initially fish use the enzymes and transporters associated with special mitochondrion-rich cells (MRCs) on their skin to move ions into and out of their bodies. However, as the fish's ion regulation requirements exceed the capacity of the MRCs on the skin and the surface area of the fish's body is too small to accommodate further MRCs, the site of ion regulation moves to the high surface area gills. Given that both gill duties – ensuring that the proper ions enter and leave the body, and O_2 extraction – are crucial to the fish, Clarice Fu and her colleagues from Universities in Canada and Portugal developed a hypothesis to directly test, for the first time, which function needed the gills to develop first.

Fu and her colleagues selected trout embryos for the study and built specialized chambers so that the newly hatched larvae's heads could be isolated from their bodies, to enable them to compartmentalize the duties of the gills and the skin. At various points in development, which they tracked as 'days post-hatch', the team measured the fish's O_2 extraction and ion (Na^+) uptake rates from the water. Because they had isolated the head (gills) from the body (skin), they could determine the proportion of each job that was performed by each body part. When the proportion of the job performed by the skin decreased and the proportion performed by the gills increased, they would know that it was time for the gills to take over the function from the skin.

The team determined that O_2 extraction transitioned from a job that was performed predominantly by the skin to one undertaken by the gills 23 to 28 days post-hatch. However, this transition occurred earlier for ion regulation; the job site switched from the skin to the gills at 15 to 16 days post-hatch. This indicated to the team that ion regulation was a bigger issue to a developing fish: the development of gill structures for ion regulation is required earlier than for O_2 extraction. Fu and her team found that this trend remained when experiments were performed in ion-rich hard water and ion-poor soft water, suggesting that the timing of gill development is intrinsic. The team's conclusions regarding gill development give scientists an insight into the evolution of primitive fishes and the kinds of fishes that may have existed. It may have been that the invasion of freshwater was what gave rise to gills, more so than a dire need for O_2.

10.1242/jeb.036509

Fu, C., Wilson, J. M., Rombough, P. and Brauner, C. J. (2010). Ions first: Na^+ uptake shifts from the skin to the gills before O_2 uptake in developing rainbow trout, *Oncorhynchus mykiss*. *Proc. R. Soc. B* doi:10.1098/rspb.2009.1545

Jodie L. Rummer
University of British Columbia
rummer@zoology.ubc.ca

TESTOSTERONE

STEROIDS GIVE LIZARDS A FIGHTING EDGE

It is said that ‘all is fair in love and war’. That is certainly the case in the animal kingdom where competition for resources is fierce. Animals compete for food, territories, shelter and mates in an effort to survive, and most importantly, to reproduce. The Tenerife lizard (*Gallotia galloti*) is no exception. Males battle violently for mates and bigger, faster and stronger males are usually the victors. But what are the proximate mechanisms driving the evolution of these traits? A potential mechanism is the effect of testosterone on physiological and morphological characteristics that will ultimately affect the performance of a male during combat. Kathleen Huyghe and her colleagues attempted to answer whether this is the case by determining how certain phenotypic traits are affected by testosterone levels.

The team collected blood samples from Tenerife lizards and determined their initial testosterone levels. They also determined several morphological characteristics such as head length, height and width, as well as two indicators of performance: maximal bite force capacity and maximum sprint speed. Although the authors did not find a correlation between the initial testosterone levels and any of the morphological traits, they did find a positive correlation between initial testosterone levels and bite force capacity. In 2005 Huyghe and her colleagues observed that bite force is a good predictor of the outcome of a battle between Tenerife lizard males. This, together with their findings that testosterone levels affect lizards’ bite force, strongly suggests that intrasexual selection is driving the evolution of these traits. On the other hand, sprint speed was not correlated with testosterone level, an interesting observation since sprint speed is not important during male–male interactions.

After obtaining the basal morphological and performance measurements, the team artificially manipulated some of the lizards’ testosterone levels by implanting them with crystalline testosterone; this type of implant allows the release of the steroid over the course of several months. About 5 months later they measured the morphological and performance parameters one more time as well as the size of the lizards’ penises and of several muscles such as the jaw closer muscles and one of the jaw opener muscles. Testosterone induced a dramatic increase in the jaw closer muscles, which provide the bite force, but not in the jaw opener muscle. However, to the team’s surprise the steroid did not affect biting force itself. The authors suggest that this outcome may be explained by a lack of muscle training, since the lizards were captive during the experiments and thus devoid of challenges they would normally experience in the wild such as fights or the necessity to crush tough foods. Lastly, testosterone caused an increase in penis size. Although it is unclear how penis size may increase fertilization success, Huyghe proposes that bigger penises may facilitate copulation despite female resistance, provide an advantage in postcopulatory sperm competition or play a role in female choice.

The results from this study suggest that testosterone is an important mechanism modulating morphological and performance traits fundamental for the reproduction of this lizard; traits which in turn can be favoured by sexual selection. As it turns out, it appears that steroids give male lizards the edge they need to attract a female, and what male wouldn’t want that!

10.1242/jeb.036525

Huyghe, K., Husak, J. F., Moore, I. T., Vanhooydonk, B., Van Damme, R., Molina-Borja, M. and Herrel, A. (2010). Effects of testosterone on morphology, performance and muscle mass in a lizard. *J. Exp. Zool.* **313A**, 9-16.

Viviana Cadena
Brock University
viviana.cadena@brocku.ca

PROPRIOCEPTION

WHERE IS YOUR ARM?

In movies and tabloids, a ‘sixth sense’ is a magical ability to sense things the rest of us can’t. But, in fact, each of us really does have a sixth sense that’s so basic we often overlook it. It’s called proprioception, and it’s what allows us to know the position and orientation of our bodies and limbs, even when we can’t see them.

Researchers have studied the various sense organs that contribute to the proprioceptive sense – including stretch receptors located in muscle bodies, force sensors in tendons and nerve fibers in joint capsules – but we know relatively little about how the brain combines all of that information. In particular, how do you know where your hand is? Does the brain track hand position directly, or does it keep track of the elbow and shoulder angles separately?

To examine these questions, Christina Fuentes and Amy Bastian at Johns Hopkins School of Medicine enlisted a group of 10 subjects. Each subject was strapped into an exoskeletal robotic arm that allowed the researchers to measure and control the angle of the subjects’ right shoulder and arm and the position of their fingertip. They placed a screen above each subject’s arm to block the view of the arm.

Fuentes and Bastian then asked the subjects to do three tasks, each of which required the subjects to estimate their forearm angle. In the first task, the researchers used the robot to move the subjects’ forearm to a particular angle, then asked them to use a joystick to rotate a line to match their forearm angle. Second, after the robot moved the arm again, the subjects used the joystick to move a point to the location of their index finger. Since the elbow was fixed, this task also amounted to estimating the forearm angle, but the subjects were thinking about the fingertip position. In the last task, rather than estimating an angle imposed externally by the robot, the subjects actively moved their arms to match the angle of a projected line.

All subjects had the largest errors in the first task, when the robot moved their arm and they were asked about their forearm angle. Interestingly, they performed much better on the second task, even though the only difference between it and the first was that they were asked about fingertip position rather than forearm angle. And they were slightly more precise in the active task, when they had to move their arm to match a displayed angle.

They attribute the differences in precision across the tasks to two effects. In the active task, in which the subjects were most precise, they could use both proprioceptive information and information about how they themselves moved their arms. The accuracy in this task wasn't all that surprising since it's fairly unusual that something else moves our limbs for us.

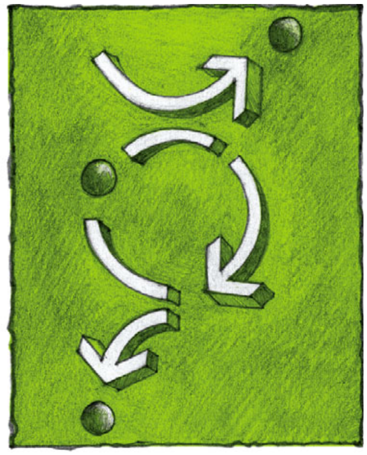
The differences in the two passive tasks is more informative. It suggests that the brain is better able to gauge hand position than actual joint angles, which makes sense behaviorally. After all, when you reach for a cup of coffee without looking, the thing that really matters isn't your elbow, but your hand position.

10.1242/jeb.036491

Fuentes, C. T. and Bastian, A. J. (2010). Where is your arm? Variations in proprioception across space and tasks. *J. Neurophysiol.* **103**, 164-171.

Eric Tytell
University of Maryland
tytell@umd.edu

NUTRITION



EAT LESS, LIVE LONGER

You are what you eat! This popular sentence coined by nutritionists in the last century refers to the simple truth that the food we eat has some bearing on our health and body. More than that, scientists substantiated the link between food and health by showing that a balanced, but restricted, diet can even extend lifespan in a wide variety of organisms. Yet, the benefit of an extended lifespan seems to be at the expense of fecundity as poorly fed organisms produce fewer offspring. The maintenance of vital body functions and reproduction appear to compete for limiting resources. Investigating this phenomenon, a team of British scientists led by Linda Partridge have shown in a recent *Nature* paper that this view is not entirely correct, as a single amino acid can fully restore the fecundity of long-living fruit flies.

Dietary research over the last few years has shown that rich diets shorten lifespan because of a dietary imbalance rather than excess calories. Moreover, experiments performed in flies and rodents indicated that lifespan and fecundity depend on the specific nutrient content of the diet, and certain amino acids appear to play a pivotal role. Partridge and her team went on to dissect this phenomenon in more detail in the fruit fly *Drosophila*. Feeding insects chemically defined diets, the scientists identified specific nutrients that affect lifespan and fecundity of the flies. The addition of amino acids shortened lifespan and increased egg laying.

The next logical step was to identify which amino acids account for the effects on lifespan and fecundity. The team found that the addition of 10 essential amino acids increased the insect's fecundity while reducing their lifespan as much as if the insects were fed on a full balanced diet. So the amino acids were responsible for the increased fertility and reduced lifespan, but which ones?

Next the scientists narrowed down the responsible amino acids by feeding the insects on a restricted diet while adding back 9 of the 10 essential amino acids, leaving out methionine. This time they could not increase the insect's fecundity, but when they added methionine alone to the diet of flies on a restricted diet the flies' fecundity was returned to normal. So the addition of methionine is sufficient to increase fecundity.

However, methionine is not the only amino acid that is necessary to reduce life expectancy. Other amino acids in combination with methionine are required to shorten life expectancy because: reintroducing methionine to the restricted diet did not shorten the lifespan; and addition of an amino acid mixture that lacked only methionine did not shorten the insect's life span either. So, in addition to methionine, one or more further essential amino acids are necessary to shorten lifespan.

Having established that methionine is involved in shortening life expectancy, the team wondered how methionine affects lifespan and found that the insulin/insulin-like growth factor signaling (IIS) pathway mediates the effects of amino acids on lifespan and fecundity.

Partridge and her team have clearly demonstrated that the extension of lifespan observed under dietary restriction is due to an amino acid imbalance in the diet – at least in fruit flies. The possibility that even mammals might profit from suitably balanced diets, maintaining the benefits of dietary restriction on longevity without the drawback of reduced fecundity, is very exciting.

10.1242/jeb.036517

Grandison, R. C., Piper, M. D. W. and Partridge, L. (2010). Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila. Nature* **462,** 1061-1064.

Hans Merzendorfer
University of Osnabrueck
merzendorfer@biologie.uni-osnabrueck.de