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

SPIGGIN: A STICKY TOXICITY INDICATOR

Endocrine-disrupting chemicals are natural or man-made chemicals that can mimic endogenous hormones and cause physiological disturbances. Released into the aquatic environment, from sources such as sewage treatment plants and pulp mills, these compounds are a perceived problem for both animals and humans as they can interact with normal hormonal function. In particular, interference with sex hormones is known to affect organisms undergoing early gonadal development and sex differentiation. In their *Environmental Toxicology and Chemistry* paper, Katsiadaki and colleagues continue to test and develop a novel method used to detect exposure of fish to androgenic compounds, based on the physiological effects of male sex steroids in female sticklebacks.

The majority of research into endocrine-disrupting chemicals has focused on oestrogenic chemicals that mimic the female sex hormones. Less is known about the chemicals that mimic male sex hormones. But while the debate rages as to the degree of impact these chemicals are having on our environment, it is generally accepted that these endocrine-disrupting chemicals are ubiquitous contaminants of the aquatic ecosystem.

Spiggin is a glycoprotein glue, which male sticklebacks use to stick together their nests. The protein is produced by the males during the breeding season in response to endogenous androgens that cause the epithelial cells of the kidney to increase in size and synthesise the protein. Histological examination of kidney epithelia cells in response to artificial administration of androgens has been used previously to monitor the physiological effects of androgens, and as an indicator of spiggin production, but this is a time-consuming method.

The authors of this study aimed to test whether direct measurements of atypical production of spiggin in female sticklebacks could be used as an indicator of exposure to environmental androgens. Recently, the team developed a spiggin enzyme-linked immunosorbent assay (ELISA), which allowed them to determine the levels of spiggin in the kidney. They wondered whether this new method could be used to detect waterborne androgen exposure in a dose-dependent manner.

They found that spiggin detection could be used successfully as an indicator of androgen exposure in female sticklebacks. Spiggin production in females exposed to increasing concentrations of the androgens 17α-methyltestosterone and 5α-dihydrotestosterone was strongly correlated with kidney epithelia cell height, but measurement of spiggin was a much quicker method with a considerably higher response range.

This work is noteworthy for two reasons. Firstly, the authors, in combination with their previous research, have successfully developed a novel and practical method of detecting exposure to environmental androgens *in vivo*. Secondly, the study clearly illustrates how contamination of the aquatic environment with endocrine-disrupting chemicals can have profound effects on the physiology of the organisms that live there and how these physiological changes can be used to develop novel methods for environmental monitoring.

10.1242/jeb.00186

Katsiadaki, I., Scott, A. P., Hurst, M. R., Matthiessen, P. and Mayer, I. (2002). Detection of environmental androgens: a novel method based on enzyme-linked immunosorbent assay of spiggin, the stickleback (*Gasterosteus aculeatus*) glue protein. *Environ. Toxicol. Chem.* **21**, 1946-1954.

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CIRCADIAN CONTROL

FROM DUSK TILL DAWN: DIURNAL RHYTHM OF NEUROGENESIS IN LOBSTERS

Life-long addition of neurons occurs in parts of the nervous systems of both vertebrates and invertebrates. Although the functional meaning of adult neurogenesis is not known in most cases, there is considerable interest in understanding the mechanisms of adult neurogenesis, with the hope of finding new treatments to heal damaged nervous tissue. Adult neurogenesis might also function in learning and memory, since one of the systems where it is found is the hippocampus of mammals. The timing and rate of adult neurogenesis appears to be controlled by a wide range of mechanisms, such as hormonal cycles, physical activity and social interactions. In a recent paper in the *Journal of Neurobiology*, Barbara Beltz and her colleagues at Wellesley college show that the proliferation of nerve cells can also be under circadian control.

Some groups of neurons in the olfactory pathway of the American lobster (*Homarus americanus*) continue to proliferate throughout the lobster's life, so the authors decided to investigate circadian control of neurogenesis in the lobster's olfactory lobe. Beltz's team used a simple protocol to monitor cell proliferation. They labeled newly developed cells with bromodeoxyuridine (BrdU), which is incorporated into DNA in place of thymidine during mitosis and can be detected by antibody-staining. The team placed juvenile lobsters in seawater that contained BrdU for 3 h periods at different times during an artificial light/dark cycle and then counted the newly proliferated cells that carried the BrdU label.

The authors found dramatic differences in the numbers of neurons that proliferated over the 3 h incubation period, depending on the point in the artifical light/dark cycle when the lobsters were incubated with BrdU. The highest numbers of new neurons were found when the incubation took place around the artificial dusk, while minimal numbers were found around dawn.

Beltz and her team also investigated the nature of the diurnal signal that caused this effect by altering the animals' light/dark cycle. The animals were kept in a normal 12 h:12 h light/dark cycle for 2 weeks before they were moved into complete darkness for 3 days. These animals retained the diurnal variation of neurogenesis, so the underlying biological rhythm appears to be endogenous. The team also found that this endogenous rhythm can be resynchronized by exposure to light. They tested a second group of lobsters that were kept in a reversed light/dark cycle and found that the rhythmicity of neurogenesis was also shifted by 12 h, so that the number of new cells still peaked around the subjective dusk and minimal counts were still found around subjective dawn.

Most animals show activity patterns tightly linked to a diurnal cycle, so hunting, feeding or social interactions are dependent on the time of day. It is also known that hormonal levels can be under circadian control. Hence, most factors that have been found to regulate neurogenesis in the adult nervous system are themselves controlled by diurnal rhythms. It is not clear to what extent circadian regulation of neurogenesis acts through these factors or if it has a more direct effect. However, the time of day could be an important cue for regulation of nerve cell proliferation in many systems.

10.1242/jeb.00187

Goergen, E. M., Bagay, L. A., Rehm, K., Benton, J. L. and Beltz, B. S. (2002). Circadian control of neurogenesis. *J. Neurobiol.* **53**, 90-95.

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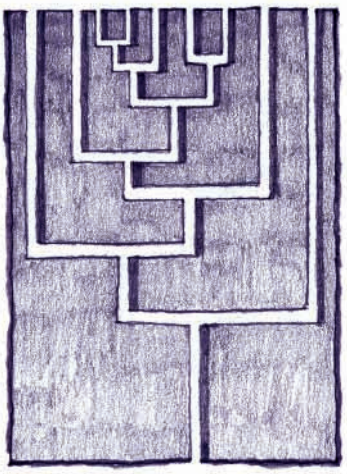
MOUSE GENOME



THE MOUSE GENOME: A FUTURE FOR COMPARATIVE PHYSIOLOGY?

The similarities between the mouse and human genomes are striking: 2.5 Gb, cf. 2.9 Gb; around 30 000 protein-coding genes; about 80% of mouse genes have a single identifiable human orthologue, and fewer than 1% of mouse genes appear to be truly unique. Indeed, despite over 100 million years of evolutionary divergence, there remain huge regions of synteny (long stretches of homologous genes in a similar order) between various mouse and human chromosomes. Of 1022 human diseases that had been mapped to genes, 807 had clearly identifiable mouse homologues. Taken together, these facts hammer out the predictable assertion that the mouse is the obvious model organism for biomedical research. Obviously, this will also direct the focus of funding agencies worldwide increasingly toward mouse. At first sight, this may not seem like good news for 'curiosity-directed' comparative physiology.

However, the following paper in *Nature* by the Mouse Genome Sequencing Consortium cruelly exposes the phenotype gap. The mouse transcriptome (all the mRNAs encoded by the genome) was mapped by a huge project, in which random cDNAs were sequenced at high volume, then clustered into groups corresponding to single genes. This is estimated to have 'hit' at least 90% of mouse genes and, in so doing, identified 33 409 genes. However, while most of these can be assigned broad functions based on similarity to known protein classes ('G protein', 'ABC transporter', etc.), relatively few of them have been named or investigated in any depth.

Functional genomics is defined as the elucidation of gene function in a genomic context or, in other words, finding out what all these genes do. In mouse, there is plenty of work to be done: only about a third of genes have been studied. But who is to work out the function of all the novel genes? Mouse has previously not been considered a major physiological model. This is the key: the mismatch between genetic and physiological understanding of an organism is called the 'phenotype gap'. Put simply, the genome projects are crying out for physiologists who are both competent and interested in the genetic model organisms, like mouse, fly and worm. The 'package' of experimental resources on offer is tempting: the availability of a sequenced genome, removing the need to hunt for new genes by homology; the free availability of cDNA clones corresponding to any of the genes described above, by post; the availability of comprehensive, ready-made microarrays covering the whole transcriptome; and the ability to address the simple physiologist's question 'what does this gene do?' by creating a knockout and seeing what happens ('reverse genetics').

Does this genome-linked future mean the end for curiosity-led research? Not at all: there is no better time to pose the higher-level 'integrative' biology questions about responses to environmental stress, homeostasis, circadian clocks or neural function.

Does the availability of the mouse genome compel scientists to work on mouse? Not at all – the very low number of mouse 'unique' genes shows that, with human and mouse genomes available, it is possible to triangulate rather accurately on any gene in any closely related species (certainly among the mammals). It is thus possible to get appreciable leverage from a phylogenetically close genome project, without necessarily working in that organism.

10.1242/jeb.00228

Mouse Genome Sequencing Consortium (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520-562.

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PARASITES PREFER HUNGRY FLIES

The parasite *Leishmania major* infects many vertebrates including humans, dogs and rodents. In humans, infection can cause skin sores and boils, fever, organ damage and even death. *Leishmania* is transmitted to humans *via* biting sand flies from Central and South America, Africa and Asia. Although flies suffer poor health when carrying the parasites, infected flies are common in these areas. Does this mean that there is some advantage associated with *Leishmania* susceptibility in sand flies?

Yosef Schlein and Raymond Jacobson at the Hebrew University in Israel recently tested this idea. As hunger-tolerance is an advantage to flies, the scientists wondered if this tolerance was somehow related to the flies' susceptibility to infection. Sand flies have a voracious appetite for plants that are packed with nutritious sugars. But by the end of the dry season, plant sugars are in short supply so that flies that can 'do without' have a real advantage. A low sugar diet limits the life expectancy of sand flies and few survive long enough to deposit their eggs and transmit their parasites. So, the flies that are best able to 'do without' are therefore most likely to produce offspring and to spread infection. Could these hunger-tolerant flies be targeted for *Leishmania* susceptibility?

The researchers investigated this possibility by exposing flies to different nutritional conditions, and infecting survivors with *Leishmania*. Flies that survived sugar-deprivation were far more likely to carry parasites than those with unlimited access to sugars. Hunger-tolerant flies were therefore most susceptible to infection. And that's not the end of the story. Schlein and Jacobson also showed that offspring from hunger-tolerant flies were just as prone to infection as their parents!

The researchers then investigated how hungry sand flies fared in the wild. They found that flies living in arid areas had empty bellies, whilst those living in irrigated sites were stuffed full of plant sugars. Offspring descended from arid area flies were much more susceptible to parasites than those from sugar-saturated parents. Again, their findings indicate a link between hunger-tolerance and *Leishmania*-susceptibility.

This study ultimately suggests that in sand flies, the success of *Leishmania* infection reflects the ability of some flies to survive periods of sugar deprivation. This association probably developed because hardy flies stand a better chance in reproduction and parasite transmission. The reason why hunger-tolerant flies are more susceptible to *Leishmania* will likely prove an appetising topic for future study.

10.1242/jeb.00188

Schlein, Y. and Jacobson, R. L. (2002). Linkage between susceptibility of *Phlebotomus papatasi* to *Leishmania major* and hunger tolerance. *Parasitology* **125**, 343-348.

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ADAPTATION

HAEMOGLOBIN WITH A(L)TITUDE

Haemoglobin is about the most thoroughly studied molecule larger than water, but it still turns up surprises. The job of delivering oxygen around the body is a tricky balancing act, and the balance depends on whose body, and where it is. Recently, Roy Weber and co-workers have shown that haemoglobin from the frog species *Telmatobius peruvianus*, which lives in snow melt at altitudes above 3500 metres in the Andes, has exceptionally high oxygen affinity.

In itself this is not unexpected – some bird species can fly at three times that altitude thanks to high oxygen affinity haemoglobin variants – but the frog has evolved rather differently. Since the classic studies of Perutz on human haemoglobin, it is well understood that the oxygen affinity of the protein can be altered radically by gaining or losing bonds that stabilise the preferred conformations of the deoxy and liganded states. Even very minor changes can affect the equilibrium between these T and R states significantly. For many animal haemoglobins, the low affinity T conformation is bound more strongly by heterotropic (non-oxygen) ligands, including organic phosphates such as diphosphoglycerate (DPG) or ATP, chloride and hydrogen ions, and Nature has found that DPG provides opportunities for fine-tuning the properties of the protein. Mammals living at high altitude tend to have haemoglobins that bind DPG more weakly than those close to sea-level, and the amino acid sequences of the proteins show alterations around the DPG binding site, between the two beta chains and in the central cavity of the tetramer. Weaker heterotropic ligand binding means the T state is less stabilised, and oxygen affinity is higher.

The first surprise with the new analysis of the frog haemoglobin is that its oxygen binding is almost completely independent of chloride concentration, the first time this means of altitude adaptation has been found. The second surprise is the protein sequence; although about 56% of the residues are identical to those of human haemoglobin, none of the usual suspects, the positively charged beta chain residues in the central cavity, are altered. This result appears to conflict with Perutz's view that chloride functions through general electrostatic effects within the protein, rather than binding at discrete sites. Instead, alpha chain residues Val 1 and Ser 131, implicated by other studies in chloride binding, are found to have mutated in the *Telmatobius* protein. Reanalyzing the sequences of other high-altitude mammalian haemoglobins (and human fetal haemoglobins) in this light suggests that they too may have weakened chloride effects. Whether or not this particular twist in the tale of haemoglobin belongs solely to a little frog living in mountain streams, or whether we used it before we were born, will be interesting to find out.

10.1242/jeb.00185

Weber, R. E., Ostojic, H., Fago, A., Dewilde, S., van Hauwaert, M.-L., Moens, L. and Monge, C. (2002). Novel mechanism for high-altitude adaptation in hemoglobin of the Andean frog, *Telmatobius peruvianus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R1052-R1060.

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