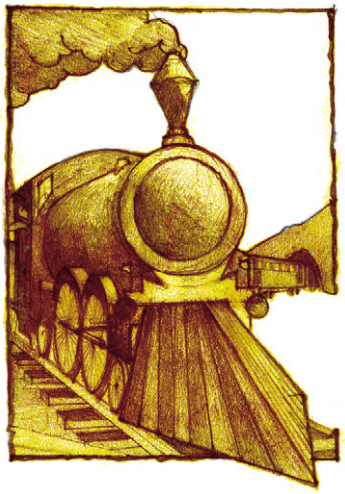
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

GAIT CONTROL



ONE PROGRAM FOR WALKING AND RUNNING

As people go down the street, they settle naturally into a comfortable walking pace. As they walk a little faster, nothing changes dramatically – their stride rate increases somewhat and several leg muscles contract more strongly – but if they speed up a little further, they eventually shift into a running gait, with an abrupt decrease in the time their feet touch the ground and a large increase in the maximum forces. Mechanically, the two gaits are very different. But is the gait transition really as discrete for the brain as it appears to be visually? Maybe one 'program', encoded in the brain and spinal cord, controls both, and the switch is due to some external cue, such as sensory feedback from the higher forces at high speeds. Or maybe running and walking are controlled by two entirely separate programs.

Answering this question requires some fancy math. G. Cappellini, Y. Ivanenko and their colleagues at the Scientific Institute Foundation Santa Lucia in Rome have been working for several years with a technique that lets them simplify the complex activation patterns of large numbers of muscles into a few relatively simple groups that act together. In their current study, they examined the activity of 32 muscles in the right legs, trunks, and right arms of walking and running humans. The researchers had their subjects run and walk at normal comfortable speeds, but also had them walk extra quickly and run very slowly, so that they had data for both gaits at the same speeds. Then, because the stride rate is faster at high speeds, they scaled all the times during a step when different muscles were active, relative to when the right foot hit the ground. Finally – this is the fancy bit – they ran the activity data through a statistical procedure called principal components analysis, which reduced the massive data set into activity patterns for five groups of muscles that acted together in consistent ways and found that the same five groups work together in about same way during both walking and running.

Each group of muscles showed an activity peak somewhere during the step cycle. For example, the hip and knee extensor muscles act together just after the foot hits the ground, and various muscles in the trunk turn on together as the leg begins to swing forward. Four of the five activity peaks showed up at the same time at the same speed, whether the subjects were walking or running. In both gaits, the peaks shifted earlier in the step cycle as the speed increased. But there was one rogue group which appeared to do different things in the two gaits at first sight; group number two, mostly muscles that point the toe, turned on late in the step during walking but early during running, suggesting that different programs were controlling that group.

Or so it appeared at first. Actually, it depends on how you define 'early' and 'late.' When the researchers looked relative to when the toe comes off the ground, then the anomalous group two patterns lined up for both gaits. The toe pointing muscles tend to come on at a consistent time just before lift off, unlike the other groups, which seem to be timed relative to heel strike.

Since all groups for both gaits are consistent relative to something, it appears that one program probably controls both gaits. The key difference between walking and running, once they get started, seems to be sensory information about the step cycle, not something centrally controlled.

10.1242/jeb.02497

Cappellini, G., Ivanenko, Y. P., Poppele, R. E. and Lacquaniti, F. (2006). Motor patterns in human walking and running. *J. Neurophysiol.* **95**, 3426-3437.

Eric Tytell
University of Maryland, College Park
tytell@umd.edu

GROUP BEHAVIOR

LOCUST MARCHING ORDERS

Strange things happen when individuals aggregate. That is, a solitary sparrow does not reveal how large flocks move. Likewise, fish and schools; bison and herds; ants and anthills; people and maddening crowds; and cars and traffic jams. Recent work, from physics, has focused on similarities across these systems. The result is a class of models based on so-called 'self-propelled particles,' in which individuals power themselves and interact with neighbors according to a set of rules and over some distance. A key prediction is that as density increases, the group undergoes a rapid transition from disorganized movement to highly ordered collective motion. In physical terms, the group should undergo a phase transition, much like water molecules do when they align during freezing.

Is this prediction supported by data? An international group, led by Jerome Buhl of the University of Sydney, tested the prediction using the desert locust, *Schistocerca gregaria*, the most widespread and devastating of the plague locusts. The insect's secret lies in a remarkable Jekyll-and-Hyde duality. Individual juvenile locusts (called nymphs) normally are, like Dr Jekyll, shy and retiring. But if compelled to socialize, for example by competition for scarce food, an individual can rapidly transform into a wild Mr Hyde (a process called gregarization), who prefers the company of other locusts and may join together with millions of his fellows to form roving bands of destruction. Band formation, however, requires that locally gregarized groups move in a coordinated fashion into other areas from which they recruit more locusts. In locust terms, the key questions are: do locusts undergo a rapid transition from random to ordered movement with increasing density and what is the critical density?

Buhl and colleagues put different numbers of locusts (5–120) into a ring-shaped arena and filmed their motion for 8 hours. The team then processed the movies with software that kept track of the orientation and movement of each individual, and found that locust movement depended dramatically on density. At low densities (2–7 moving locusts) individuals were rarely aligned. At medium densities (10–25 moving locusts), individuals were highly aligned for most of the 8 h test period, but showed rapid and spontaneous changes in direction. At high densities (30 or more locusts), the locusts almost immediately became aligned and did not change direction thereafter during the test period (see online movie). These results conformed closely to the output of simulated 'self-propelled particle' models encoded with known locust biology, suggesting that the locust experiment represents a special case of a more general process influencing the movement of other animal aggregations.

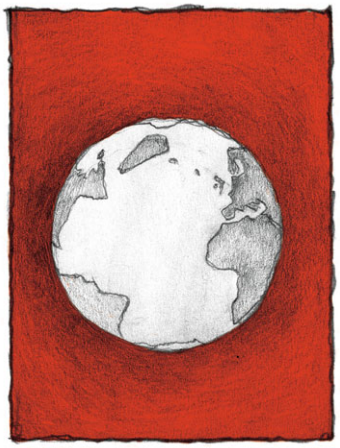
The team's work sheds welcome light on a devastating insect problem. Previous field work has shown that the lower density in marching bands is about 20 locusts per m^2, which corresponds to 8 locusts in the ring-shaped arena. Buhl and colleagues' experiment confirms this threshold density but also adds interesting, and sobering, detail. Above the threshold, locusts are almost always aligned although medium-density locusts often change direction while high-density locusts do not; they seem to have more directional inertia. This result suggests, counter-intuitively, that the movement of high-density swarms may be easier to predict and control.

10.1242/jeb.02498

Buhl, J., Sumpter, D. J. T., Couzin, I. D., Hale, J. J., Despland, E., Miller, E. R. and Simpson, S. J. (2006). From disorder to order in marching locusts. *Science* **312**, 1402-1406.

Art Woods
University of Montana
art.woods@mso.umt.edu

HYPOXIA



RESETTING THE OXYGEN-SENSING SET POINT

A significant drawback to living in air is that there is always a risk of death from metabolic failure if oxygen levels fall too low (hypoxia), and from oxidative damage if O_2 gets too high (hyperoxia). Much recent attention has focused on the cellular mechanisms that protect us from oxygen damage, as well as the sensors that trigger the responses, but a recent study by Savita Khanna and colleagues in *Free Radical Biology and Medicine* approaches the question from a different angle: how does a cell decide what are normal and abnormal oxygen levels and can it reset the 'normal' level?

All aerobic organisms have developed elaborate adaptive mechanisms to maintain oxygen homeostasis, but in order to control oxygen levels the organisms must first detect it before initiating a signalling cascade to induce the appropriate survival responses. One such survival response is an increase in HIF (hypoxia inducible factor). HIF regulates more than 60 putative target genes involved in hypoxia tolerance *via* a heat shock response element (HRE). During normoxic conditions, HIF is continuously degraded by an oxygen-dependent process. The enzyme proline hydoxylase (PHD) initiates the degradation by hydroxylation of the transcription factor's proline residues. When oxygen levels decline, HIF is no longer degraded so the protein levels rise, ready to upregulate genes involved in protecting the organism during hypoxia. As tissue oxygen levels vary widely within an organism, from organ to organ and cell to cell and even within a cell, oxygen sensing must be a flexible and highly adaptive system. However, instead of focusing on the O_2 sensor itself, Khanna and colleagues decided to investigate how a cell decides what its 'normal' oxygen pressure (P_{O_2}) is by testing the hypothesis that cells are

capable of resetting their normoxic set point by changing expression levels of proline hydoxylase.

The team used a murine neuronal cell line (HT22 cells), which they transfected with a luciferase reporter gene; this expressed light-emitting luciferase when HIF bound the heat shock response element. Using this HIF indicator, Khanna and colleagues tested cellular responses to hypoxia; hypoxia should increase HIF levels and induce clearly visible luciferase expression. Cells exposed to 5% O_2 (instead of the 20% O_2 cell cultures are generally grown in) showed significantly higher HRE-driven luciferase expression, with further luciferase activity induced when O_2 levels were decreased to 0.5%. However, cells maintained at 5% O_2 for 4 weeks no longer increased HIF activity in response to that P_{O_2}, and when the oxygen levels was decreased further to 0.5% O_2 the resulting induction of HIF activity was significantly less than in cells adjusted to a norm of 20% O_2. This suggested that the biological response to a given P_{O_2} is not so much dependent on the P_{O_2} itself but on conditioning of the cells.

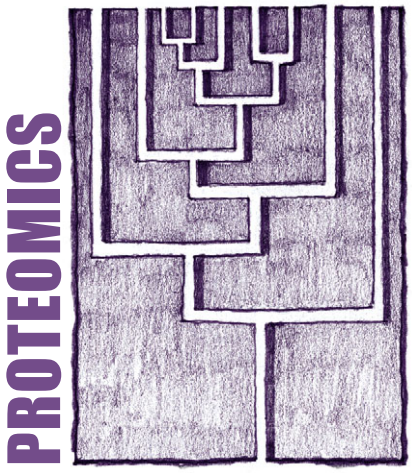
To further test the hypothesis that the normoxic set point in cells is adjustable, additional HT22 cells were grown in hyperoxic conditions at 30% O_2. Acute exposure of cells maintained in 30% to 'normal' oxygen levels of 20% resulted in increased HIF activity; the normoxic set point can thus clearly be adjusted both upwards and downwards.

The team then went on to investigate proline hydroxylase mRNA expression and found that cells maintained at low O_2 increased proline hydroxylase expression compared to the 20% O_2 cells, while decreasing proline hydroxylase expression in high O_2. They also looked at proline hydroxylase levels in mice maintained in hypoxia (10% O_2), which reduced brain P_{O_2}, and found that the animals had significantly increased proline hydroxylase expression. Taken together the data indicate that changes in proline hydroxylase expression may be effective in resetting the normoxic set point of cells, and that 'normal' P_{O_2} is clearly adjustable over a wide range of oxygen levels even within a single cell type.

10.1242/jeb.02495

Khanna, S., Roy, S., Maurer, M., Ratan, R. R. and Sen, C. K. (2006). Oxygen sensitive reset of hypoxia-inducible factor transactivation response: Prolyl hydroxylases tune the biological normoxic set point. *Free Rad. Biol. Med.* **40**, 2147-2154.

Sarah L. Milton
Florida Atlantic University
smilton@fau.edu



PITFALL OR PROMISE: PROTEOMICS FOR NON-MODEL ORGANISMS

As environmental conditions change, organisms respond by changing the expression patterns of the genes, and proteins that are encoded by their genomes, to cope with their altered circumstances. Changes in protein expression patterns in response to physiological phenomena can be tracked by two-dimensional (2-D) gel electrophoresis, where thousands of proteins from a tissue are separated on a single electrophoresis gel. However, most so-called 'proteomics studies' to date have focused on model organisms who's genomes have been sequenced and gene products identified. With this information in hand, the masses of protein fragments can be determined and matched with theoretical values in order to identify individual proteins. Kevin Russeth and colleagues from the University of Minnesota in Duluth have questioned whether this 'proteomic' approach can also be applied successfully to unsequenced non-model organisms with limited genomic information available. Specifically, they asked whether using combinations of protein search programs improves the identification of proteins in such organisms. By combining and comparing various software programs that are currently used in protein identification, they also tested the assumption that we all too often make: that we can trust the results of sophisticated software.

Russeth's team chose 7 proteins from a 2-D gel of tissues from the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) that were expressed during hibernation, as the team were curious to identify protein's involved in the animals' physiological response to hibernation. After isolating the proteins, they were digested into peptide fragments with the protease trypsin and the masses of the resulting fragments measured with high-resolution mass spectrometry. Once the team knew the masses of the peptide fragments from each protein and their amino acid sequences (or content), they analysed the mass patterns with four different algorithms, Mascot, Pro ID, Sequest and Pro BLAST, to try to identify each protein.

Surprisingly, all four programs identified the same proteins from each mass spectrum, with a minimum of 2 and a maximum of 16 unique peptides required to confidently identify a protein. However, the number of peptides that were determined to belong to an identified protein varied from program to program. Thus all the programs obtained the same results, despite differences between the search algorithms, much to the authors' relief.

But how confident can we be in the protein identifications? While several programs achieving the same identification is promising, the authors were cautious and inspected the positively identified spectra manually and judged that two of the seven mass spectra were of insufficient quality to warrant a confident identification, despite the agreement among programs.

Equipped with some assurances that we can confidently identify proteins in organisms with limited genomic information, we might expect some juicy insights into the differences in proteomic expression levels between active and hibernating squirrels, and the authors deliver well on this. They found that a mitochondrial protein, succinyl coenzyme A transferase (SCOT), became significantly up-regulated in the heart of hibernating squirrels. High levels of SCOT are likely to facilitate the increased use of ketone bodies, produced in the liver and delivered by the blood to the heart, as a metabolic fuel while the mammal is dormant.

Thus, despite potential pitfalls that continue to challenge the fully-fledged application of proteomics in non-model organisms, Russeth and his team have shown that there is much promise. It seems that a combination of high quality mass spectrometry, sophisticated search algorithms and a bit of chutzpah are essential for the successful application of proteomics to study non-model organisms.

10.1242/jeb.02496

Russeth, K. P., Higgins, L. and Andrews, M. T. (2006). Identification of proteins from non-model organisms using mass spectrometry: application to a hibernating mammal. *J. Proteome Res.* **5**, 829-839.

Lars Tomanek
California Polytechnic State University
San Luis Obispo
ltomanek@calpoly.edu

MORPHOGENESIS

DISC CONTROL

Holometabolic insects such as flies or moths have a very special life cycle. They change shape during development as they progress from larval to pupal and adult stages. The critical processes through these transitions are moulting and metamorphosis. How these processes are controlled is an outstanding question among insect physiologists. It is well known that three hormones control moulting and metamorphosis, i.e. prothoracicotropic hormone, ecdysteroids and juvenile hormone (JH). The juvenile hormone's function is to modulate the action of ecdysteroids and to determine the nature of the moult; whether the insects will pass through a larval to larval, larval to pupal or pupal to adult moult. But juvenile hormone does even more as new research shows. The husband-and-wife team, James Truman and Lynn Riddiford, together with co-workers, has recently demonstrated in *Science* that juvenile hormone suppresses imaginal disc formation and differentiation independently of the action of ecdysteroids.

Imaginal discs are primordial tissues in the larvae, which store small groups of premature embryonic cells that are ready to develop into adult organs, such as wings, legs or antennae, as soon as metamorphosis is initiated. To investigate the control of imaginal disc morphogenesis, the scientists analysed the influence of the nutritional-state and juvenile hormone levels on this process in the tobacco hornworm, *Manduca sexta*.

In the final larval stage of development, juvenile hormone levels typically decline in the insect's blood, triggering the onset of imaginal disc morphogenesis. When the scientists starved larvae, the formation and growth of imaginal discs ceased; the team concluded that juvenile hormone functions to suppress imaginal disc morphogenesis, as starvation is known to raise juvenile hormone levels. Testing the effects of juvenile hormone on imaginal disc development further, the scientists removed the *corpora allata* (the organ that secretes juvenile hormone) to reduce the hormone levels, and found that the imaginal disc developed, regardless of whether the larvae had been starved. Interestingly, control of disc morphogenesis is independent from ecdysteroids, since the placement of ligatures to generate ecdysteroid-free portions did not interfere with the development of affected discs.

The action of juvenile hormone also appears to be independent of the insect's nutrition state, as starved larvae that had lost the *corpora allata*, but received a dose of a juvenile hormone mimic, showed inhibited disc morphogenesis. Thus, juvenile hormone can function as an intrinsic signal to inhibit imaginal disc morphogenesis. However, extrinsic signals that are nutrient-dependent also seem to be involved in this process, as the imaginal disc of well-fed larvae began developing during morphogenesis, overriding the inhibitory action of juvenile hormone. To date, the precise nature of the involved factors is unknown.

Even though feeding overrides juvenile hormone suppression of imaginal disc development in the last larval stage, subsequent steps in disc differentiation are still affected by juvenile hormone. For example, insects that received juvenile hormone early in the last instar showed normal early disc differentiation, which became aberrant after the second day; growth and the number of cell divisions in the imaginal disc declined compared with control animals. Thus, juvenile hormone appears to affect two different phases of disc growth: the initial formation and growth of the disc and the subsequent differentiation of the disc into more complex structures. Most importantly, nutrition-dependent factors appear only to block the initial phase of disc formation but not subsequent differentiation steps.

In summary, the team demonstrated that imaginal disc morphogenesis is controlled by both, nutrient-dependent factors and juvenile hormone, independently of ecdysteroids. Without juvenile hormone the growth of the discs resembles that of tumors, but when juvenile hormone is present disc growth is coordinated with that of the other larval tissues. Molecules with similar properties to juvenile hormone may therefore turn out to be potent anti-tumor agents.

10.1242/jeb.02494

Truman, J. W., Hiruma, K., Allee, J. P., MacWhinnie, S. G. B., Champlin, D. T. and Riddiford, L. M. (2006). Juvenile hormone is required to couple imaginal disc formation with nutrition in insects. *Science* **312**, 1385-1389.

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