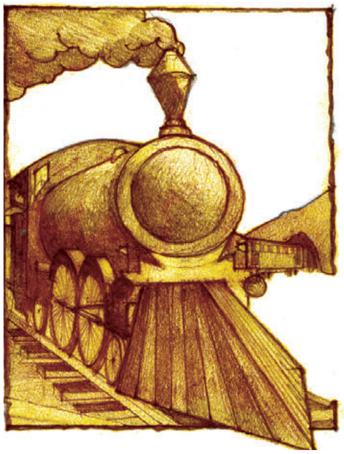
Outside JEB

MODELLING



WALK AND RUN TO BE LAZY

Why do we like to walk when we move slowly, but run when moving quickly? There are many ways of approaching such a question, but a fairly fundamental start can be made by considering the energetic requirements of gaits. Back in 2006, Manoj Srinivasan and Andy Ruina (at Cornell University) reported a computer optimisation of a ‘minimal biped model’, which searched for the energetically cheapest way of moving the body along. Despite the extreme simplicity of the ‘minimal biped model’ – consisting only of a point mass and massless legs – they (and their computer) discovered walking and running to be energetically optimal at slow and fast speeds, respectively.

But the really interesting bipeds – humans, birds and robots – do deviate quite significantly from the extreme reduction used in the 2006 computer optimisations. Does this matter? How should we walk and run if a little more realism is included? It is this that Srinivasan (now at Ohio State University) starts to approach, by sticking with massless legs and point-mass bodies, but including knees, spring tendons and a range of models for cost due to muscles.

Again, Srinivasan asked a computer to calculate many, many possible ways each biped model could possibly locomote. And then those gaits that allow locomotion at minimum energetic cost for a given speed were found.

Walking at low speeds and running at high speeds were again found. Without sensible constraints, extreme forms of ‘inverted pendulum’ walking and ‘impulsive’ running were found to be best. This means walking with a hugely forceful push-off, followed by a completely passive vault and finishing with another hugely forceful crash as the next foot hits the ground; for running, this means immensely forceful, but brief, vertical forces interspersed with passive ballistic aerial phases between each stance. Of course, neither biology nor engineering ‘likes’ infinite forces, even if they may be, according to extreme reductionist models, theoretically optimal. Adding any one of many sensible constraints or cost functions quickly makes the predicted gaits more realistic, and removes the prediction of huge leg forces.

Interestingly, these findings also stand for a model including a knee, and cost model appropriate for an electric motor (a ‘knee-torque-squared’ cost). So, the energetically sensible way for kneed robots to move is… pretty much like us; expect future C-3POs to walk with a vaulting, inverted pendulum path when slow, and run with ballistic aerial phases when fast.

Unsurprisingly, adding a lossless spring to the model allows gaits to be found that require no work from the muscles; the springs perform all the work required from the legs. However, with the inclusion of an energetic cost associated with the force (as opposed to the work) applied by the muscles, more compliant gaits again become favoured. Srinivasan also describes the family of muscle cost models – within which standard models fall – that predict walking and running, with a gait transition in between. For all these muscle cost models, the gaits minimising the cost of the body motions are mostly indistinguishable from those minimising the cost of the muscle work.

So, walking and running gaits are energetically sensible, and remain so even when further complexity is added to the model bipeds. Also, more realistic gaits (those without infinite forces) can be predicted with many of these additions. But exactly which additions are most realistic – exactly which muscle cost model? And how much tendon elasticity? This remains to be determined.

10.1242/jeb.036699

Srinivasan, M. (2010). Fifteen observations on the structure of energy-minimizing gaits in many simple biped models. *J. R. Soc. Interface.* doi: 10.1098/rsif.2009.0544.

James Usherwood
Royal Veterinary College
jusherwood@rvc.ac.uk

JIP SPECIAL ISSUE ON INSECT RESPIRATORY BIOLOGY

The respiratory systems of insects and their functions differ markedly from vertebrate respiratory systems, and have been a source of scientific curiosity for decades. Early investigations between the 1930s and 1960s inspired the development of modern sophisticated techniques that opened the field to other biologists. A brief overview of this rapidly growing field was recently published as a special edition in the *Journal of Insect Physiology*. This issue, edited by S. K. Hetz, contains contributions that reflect a dazzling diversity in insect respiration.

Lazaro Centanin and colleagues review the mechanisms of oxygen sensing that modulate and control the morphology of the *Drosophila* tracheal system, specifically the oxygen sensing mechanisms of tracheal cells *via* factors that regulate transcription factors from the HIF gene family that establish a strong developmental genetic basis for physiological responses to variable oxygen availability. In a discussion of a parallel adaptation to tracheal modulation, Christian Pick and co-authors suggest that hemocyanin (Hc) is an oxygen delivery pigment in late embryos and first-instar nymphs of an ovoviviparous cockroach.

In water boatmen (diving insects) Philip Matthews and Roger Seymour compare measured changes in gas gill volumes and their P_{O_2} during dives in water containing different oxygen concentrations with predictions from current models, and describe compressible gas gills as mechanisms to maximise gas exchange in air-breathing aquatic insects. The potentially problematic trade-offs of sufficient oxygen uptake *vs* water loss in insect eggs are reviewed by Art Woods. Using *Manduca* eggs as a model system he discusses the eggshell's role in these trade-offs during the changing metabolic demands of developing eggs.

Several papers deal with discontinuous gas exchange cycles (DGC). Thomas Förster reinvestigates the reactions of moth pupae's spiracles to gas mixtures of varying oxygen and carbon dioxide content by measuring pressure changes to detect thresholds of intra-tracheal gas concentrations which could result in spiracular fluttering. Alex Kaiser and co-authors cover the changes in intermittent gas exchange during mealworm pupal development. They investigated changes in carbon dioxide release patterns of single pupae during development and found that interburst durations changed while burst volumes remained constant as pupal development modulated metabolic rate. By contrast, John Terblanche and Steven Chown found that interburst durations remained constant while burst volumes changed as temperature changes (another factor modulating metabolic rate) in tsetse flies *Glossina morsitans*.

Heidy Contreras and Tim Bradley also used temperature as a modulator for spiracle activity *via* metabolic rate in a cockroach and a bug. Higher temperatures increased metabolic rates, and their animals changed gas exchange patterns from discontinuous to cyclic to continuous. Reet Karise and co-authors found similar temperature effects in bumblebees, showing DGCs mostly at lower temperatures or in inactive states. Christian Mörbitz also confirmed that low metabolic rates coupled with sufficient spiracular conductance are required for insects to maintain DGC. However, during periods of high metabolic demand, such as insect flight, the insect tracheal system must be capable of extreme oxygen delivery capacities. Fritz-Olaf Lehmann reviews recent studies in flying *Drosophila* and describes how convection by different, sometimes strange, mechanisms increases gas exchange capacity tremendously.

The above are only brief summaries of a few of the contributions made to this special edition of JIP that exemplify the wide range of topics currently under investigation. While we do acknowledge that technological improvements have bolstered the recent boom in insect respiration studies, it is the contributions made by researchers benefiting from these new technologies that have expanded the field of insect respiratory biology far beyond the 'breathe in, breathe out' approach that initiated the first investigations of these fascinating biological systems.

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Hetz, S. K. (ed.) 2010. Insect respiration. *J. Insect Physiol.* **56**, 445-558.

C. Jaco Klok
Arizona State University
cjklok@asu.edu

SPERM WARS: THE MOVIE

Our understanding of the mechanics of sexual selection has come a long way since 1871 and Darwin's second-most-famous book *The Descent of Man, and Selection in Relation to Sex*. At that time, the focus remained within the bounds of relatively polite pre-mating activities such as courtship rituals or duels between amorous males. However, since the 1970s researchers' eyes have been firmly drawn towards the intimate post-copulatory processes that occur inside the female body during and after mating. While our understanding of how male gametes compete in the race to fertilise eggs has advanced steadily, our understanding of the female's role in sperm selection, termed cryptic female choice, is less clear. One major barrier is that these cryptic choices happen inside the female body and are difficult to discern with any clarity. Now, in a paper published in *Science*, Scott Pitnick and his colleagues describe an extraordinary way of viewing internal processes, as they are played out within the female tract.

Sperm competition studies have long been impeded by the inability to determine which sperm belong to which male. The authors have now solved this problem by generating transgenic lines of *Drosophila melanogaster* that produce fully functioning sperm with heads that produce either green or red fluorescent protein tags so that they can be distinguished from other competitor male's sperm. The authors then filmed and tracked the progress of the fluorescent sperm of two competing males in the dissected organs of a female after mating.

As a first insight, the sperm's striking speed and manoeuvrability clearly exceeded the team's expectations. Pitnick and his colleagues also zoomed in on the fine detail of the interactions between the sperm. They saw that the ejaculate from one male displaces the sperm of predecessors from female storage organs (two spermathecae and a seminal receptacle); as a male's success is proportional to the number of sperm held in store by a female and males can effectively shift the odds of success in their favour by flushing out the competition. The team was also able to confirm the female's role in biasing fertilisation by showing that they can eject sperm from the uterus. However, there was no indication that the ejaculate of any male can damage or kill rival sperm.

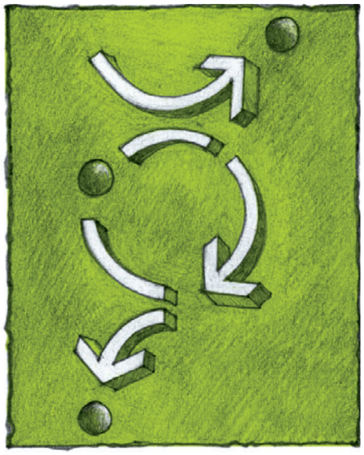
By filming the progress of identifiable sperm in the female's reproductive tract, Pitnick and his colleagues have convincingly verified (and disproved) many predictions, based on theory and indirect observations, about the hitherto unseen and complex events that occur in the female reproductive tract prior to fertilisation. Building on their success in *Drosophila*, the team are applying their approach to other insect model systems to lift the lid on the fate that befalls sperm once they enter the female. Such direct investigation could herald a golden age in the study of sexual selection, as we can at last witness sperm cells skirmishing from front row seats in real time. Moreover, the technique could help decipher the all-too-cryptic choices that females make, with the hope of resolving controversies regarding the existence or importance of sperm selection by females.

10.1242/jeb.036715

Manier, M. K., Belote, J. M., Berben, K. S., Novikov, D., Stuart, W. T. and Pitnick, S. (2010). Resolving mechanisms of competitive fertilization success in *Drosophila melanogaster*. *Science* **328**, 354-357.

Oliver Yves Martin
ETH Zurich
oliver.martin@env.ethz.ch

GENE CONTROL



FLOUR BEETLES JOIN THE GAL4/UAS CLUB

Being able to control gene expression in defined parts of an organism's body and at distinct developmental stages allows researchers to determine the functional significance of gene expression patterns. Unfortunately, the ability to do this has been limited to a few genetic model organisms. Johannes Schinko and colleagues at the Georg-August-Universität Göttingen recently tackled the task of adding a new species, the red flour beetle (*Tribolium castaneum*, which, as a beetle, represents the most species-rich metazoan taxon) to the list of model organisms in which spatial and temporal control of gene expression is possible. The team published their work in *BMC Developmental Biology*.

The team focused on adapting existing technology to their animal. The GAL4/UAS system is a widely used technique for controlling gene expression in fruit flies. It is composed of a yeast protein (GAL4) that specifically binds to a sequence of DNA known as the upstream activator sequence (UAS), triggering the expression of any gene inserted immediately behind the UAS. The choice of gene is up to the researcher. By themselves, GAL4 and UAS do nothing in a non-yeast cell, but bring them together in the same cell and together they function as an 'on' switch that activates expression of the chosen gene. Researchers then control GAL4 expression so that they can selectively switch their chosen gene on and off in specific locations and at specific times.

First, Schinko and his colleagues tried to directly translate all of these *Drosophila* techniques into beetles. They incorporated GAL4 (under the control of fruit fly promoters) into flour beetle tissues. They then put 'reporter genes' under UAS control in the same tissues. Reporter genes encode proteins (e.g. green fluorescent protein) that allow researchers to visualise when and where GAL4 successfully initiates gene expression. But all of this turned out to be a dead end. Under conditions that lead to robust GAL4 activity in flies, the researchers saw no evidence of reporter gene expression in beetles. Fruit-fly-based GAL4/UAS appeared not to work in beetles.

The team reasoned that the use of native beetle promoters might solve the problem, so they swapped a fruit fly heat shock promoter, which switches on the insect's heat shock response, for an equivalent beetle promoter. When these beetles were heat shocked, they showed robust reporter gene expression in multiple body areas, indicating that the GAL4/UAS system was functioning. The team then went on to assay how quickly they could induce this reporter gene expression in embryos. They found evidence of reporter gene activity within 2–4 hours of heat shock. This response time is only a fraction of the time needed for embryogenesis (72 h), allowing researchers to use the GAL4/UAS system for fine analyses of gene function in *T. castaneum* embryos during development. Finally, the team tested a second beetle promoter '*Tc-hairy*' and found reporter gene expression confined primarily to the central nervous system of the animal. This suggests that GAL4/UAS can also be used to spatially restrict the activity of selected genes in beetles.

Overall, the work of Schinko and colleagues lays the methodological foundations for a new era of powerful developmental and genetic experiments in flour beetles. The experiments presented are not flashy, but they are carefully done with an eye on the long-term future. Unfortunately, the importance of (and the amount of work that goes into) this type of methods publication is often under-appreciated. Ironically though, in the long run, these types of understated 'tool-building' efforts often end up being the real engines driving innovation in biological research.

10.1242/jeb.036723

Schinko, J. B., Weber, M., Viktorinova, I., Kiupakis, A., Averof, M., Klingler, M., Wimmer, E. A. and Bucher G. (2010). Functionality of the GAL4/UAS system in *Tribolium* requires the use of endogenous core promoters. *BMC Developmental Biology* **10**, 53-65.

Stefan Pulver
University of Cambridge
sp553@cam.ac.uk