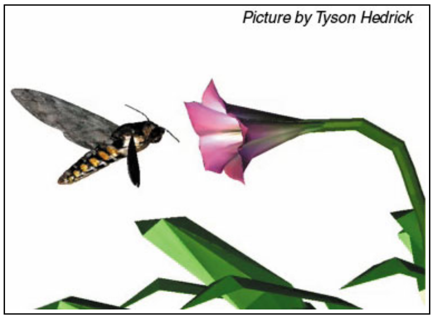
Inside JEB

Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

KEEPING MATH-MOTH ALOFT


Picture by Tyson Hedrick

When most people think of solving a problem, they start at the beginning and work through to the end. But not Ty Hedrick and Tom Daniel. When they were curious to find the range of wing beat movements that could sustain a hawkmoth's hovering flight, the team, based in Seattle Washington, realised that they'd have to think backwards and deal with hovering flight as an 'inverse problem': start with the hovering and work back to find out which combinations of wing beats could keep the moth in place above a flower (p. 3114). Tackling this problem turned out to be an enormously challenging computational task, requiring a 16-processor cluster, to discover which wing beat combinations can keep a moth hovering.

So why work on hovering and not some other manoeuvre? Hedrick explains that hovering is probably the insect's best-understood form of flight. Not only is hawkmoth hovering well characterised in the literature, but the equations that govern hovering flight are well understood and it was relatively simple for Hedrick to incorporate them into a mathematical simulation.

Having developed the mathematical moth, Hedrick chose 10 wing motion parameters, which the insects probably vary during flight, that he could adjust during the course of each simulation, and began running over 125 million simulations to see which combinations of wing beat parameters kept the moths hovering and which sent them crashing. Starting each simulation with a unique set of wing beat parameters and running it over a flight of 41 wing beats, Hedrick used a genetic algorithm to slightly modify a subset of the parameters from beat to beat, keeping the rest at their initial value, to simulate the wing beat variability inherent in the real world.

After weeks of number crunching, Hedrick and Daniel found the wing beat parameters that seemed to influence the moth's ability to hover most, including wing beat amplitude and the timing of the forward wing beat relative to the downwards wing beat. The team also found that allowing three of the wing beat parameters to vary during the course of a simulation produced the optimal hovering performance. However, increasing the numbers of variable parameters to 4 or more didn't improve the simulation's performance at all, which wasn't entirely surprising given that Hedrick and Daniel allowed their moth simulation to hover with only three degrees of freedom. The team turned up an enormous range of wing beat parameter combinations that kept 'math-moth' aloft and Hedrick suspects that 'there's a whole universe of possibilities available to hovering moths, and the real ones probably live in a small set of those possibilities'.

Hedrick admits that he didn't compare his simulated moths with the real thing until the end of the project when all the tests were run and the data were in; 'we didn't want to prejudice our results' he says. But after comparing math-moth's performance with the moths in the literature he admits that there was a 'big sigh of relief' when the simulations behaved just like the real thing.

10.1242/jeb.02452

Hedrick, T. L. and Daniel, T. L. (2006). Flight control in the hawkmoth *Manduca sexta*: the inverse problem of hovering. *J. Exp. Biol.* **209**, 3114-3130.

WET *VERSUS* DRY WEBS


Picture by Todd Blackledge

No matter how elegant or elaborate, every spider's web must successfully absorb enormous impacts as it traps and detains victims. So spiders have evolved a complex suite of web proteins each with a specific function, including stretchy structural proteins to detain passers-by in the web's spiral and glues to secure them. Todd Blackledge explains that while araneoid spider adhesives are secreted as liquid droplets on an elastic capture silk spiral, deinopoid spiders apply a more ancient dry adhesive to their capture silk; microscopically thin threads, known as cribellar fibrils, which are densely coiled around a core cable of capture silk. Curious to know how the dry cribellar adhesive impacts on the capture spiral's ability to ensnare prey, Blackledge and Cheryl Hayashi began destruction-testing spiders' webs (p. 3131).

But first, Blackledge had to convince spiders to spin webs in the lab. Having collected four cribellar spinning species from sites in Florida and near Hayashi's University of California lab in Riverside, Blackledge provided the animals with comfortable accommodation to encourage them to spin. Fortunately all three genera were content to spin their webs, but collecting the intact structures wasn't so easy. Blackledge explains that *Uloborus* spins horizontal disk shapes that were relatively easy to collect, while *Hyptiotes* and *Deinopis* actively hunt with their webs, distorting them as they trap their prey; *Hyptiotes* holds its triangular web taught, releasing it to entangle trapped victims, while *Deinopis* sits patiently overhead, ready to drop down and force its stretched web over unsuspecting passers-by. Knowing that both webs would be ruined if the spiders attempted an attack, Blackledge designed frames to capture the webs before they struck.

Having gathered the delicate structures, Blackledge collected short lengths of the dry composite spiral silk and measured the pseudoflagelliform fibres' diameter to calculate the core's cross-sectional area. Next Blackledge carefully attached the dry silk to a Nano Bionix® tensile tester and began slowly stretching it while measuring the increasing load until the silk snapped.

Calculating the dry silk's stress–strain curve, Blackledge could see that the material was relatively stiff and inelastic during the early loading stages compared to the liquid adhesive silk. However, as the load increased, the dry silk went through a transition and became permanently deformed as it extended until the core cables eventually snapped at twice their original length. Despite the broken core cables, the silk kept on stretching as the delicate cribellar fibrils remained intact until the silk had stretched up to five times its original length.

Comparing the stretchiness of the composite dry cribellate silk with capture silks coated in liquid adhesive, Blackledge realised that both silks were equally stretchy, but the dry silk's core cable was nowhere near as stretchy as the core cable from liquid coated webs. Blackledge explains that early in evolutionary history, araneoid spiders also spun cribellar silks before abandoning them in favour of less costly liquid adhesives, and he suspects that the development of stretchy core cables could have allowed the arachnids to swap wet adhesives for dry.

10.1242/jeb.02454

Blackledge, T. A. and Hayashi, C. Y. (2006). Unraveling the mechanical properties of composite silk threads spun by cribellate orb-weaving spiders. *J. Exp. Biol.* **209**, 3131-3140.

BUTTERFLY'S BLUE GENES

Most people are lucky to encounter one surprise during the course of their research, but when Adriana Briscoe began investigating opsin gene duplication in butterfly eyes, she hit the surprise-jackpot. All Briscoe knew when she began investigating the expression of photopigment genes (opsins) was that the eyes of *Lycaena rubidus* butterflies expressed four photopigments, rather than the three found in most other butterflies. Her long-time colleague, Gary Bernard, had also found that the distributions of these photopigments were different between the sexes. From this starting point Briscoe, Bernard and the rest of her team decided to clone the genes (p. 3079) to find out whether they were dealing with a gene duplication or an allele (slightly different copies of the same gene on different chromosomes).

Extracting mRNA from butterfly eyes, Marilou Sison-Mangus cloned all four butterfly eye opsin genes, and could clearly see that the extra opsin wasn't an allele; one of the other three regular opsin genes had been duplicated to give rise to the extra photopigment. But which one? Briscoe explains that insect eyes usually express one ultraviolet (UV)-sensitive pigment, one blue-sensitive pigment and a long wavelength sensitive pigment. In most cases, it's the long wavelength gene that has doubled up. But when Briscoe and the team aligned the butterfly's gene sequences, they realised this couldn't be the case. The extra gene had all the hallmarks of a blue opsin: surprise number one.

But which blue gene gave rise to which blue pigment? Knowing that the photopigments' distributions were different in the male and female's eyes, Briscoe decided to match the photopigments' locations with the gene expression patterns to identify the gene that was responsible for the extra blue photopigment. Bernard mapped the photopigment distributions and found that the only blue opsin that occurred in the dorsal regions of both male and female eyes was the opsin tuned to 437 nm. Next, Marilou Sison-Mangus painstakingly explored each gene's expression pattern with RNA probes and identified the blue genes responsible for the 437 nm and 500 nm photopigments.

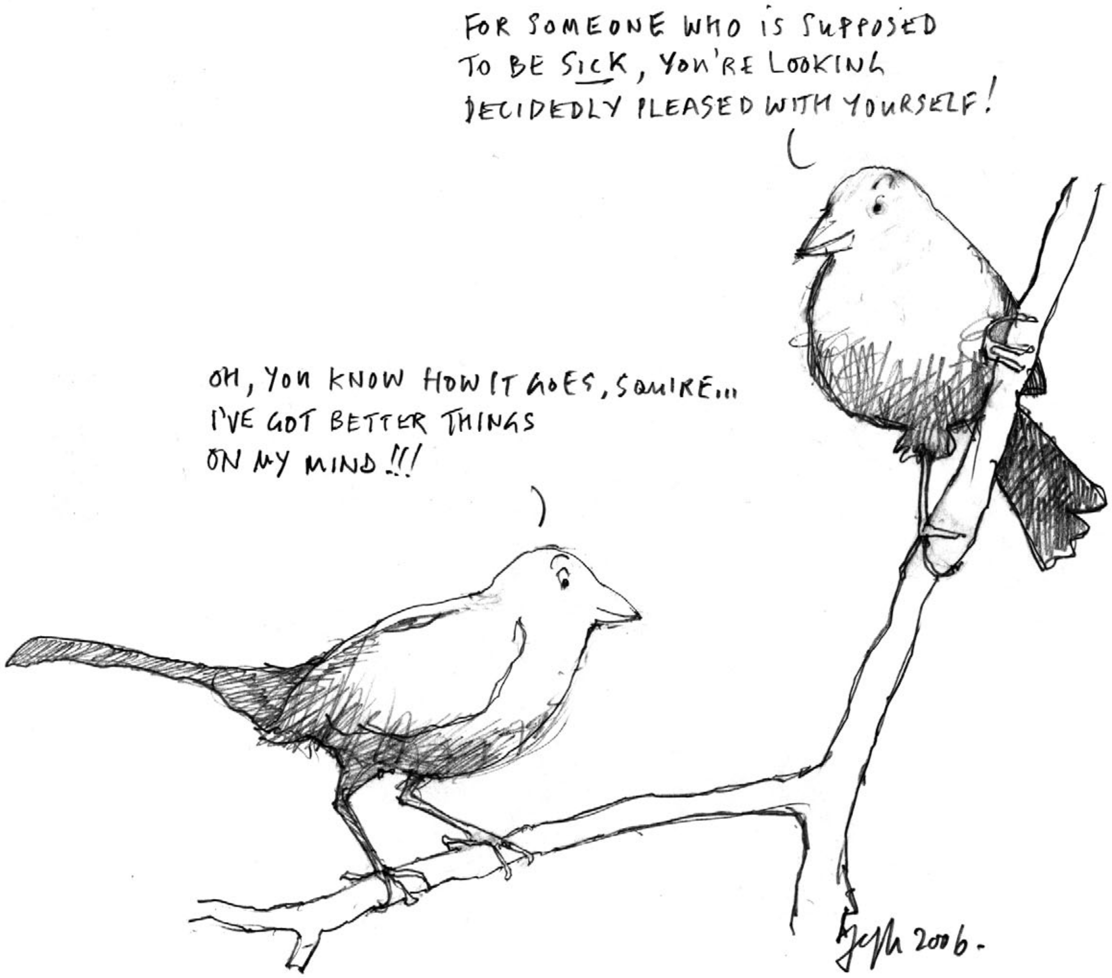
The gene mapping also threw up the second and third surprises. Firstly, the opsin gene expression patterns in the dorsal region of the male's eye were unique and unlike the patterns in any other butterflies' eyes, and secondly, some visual receptors in the dorsal region of the female's eye expressed two opsins simultaneously in a single cell. No one had ever seen a receptor cell expressing both blue and long wavelength opsins before; usually they only express one.

Finally, Briscoe explains that in most butterfly eyes each ommatidium is composed of 9 photoreceptor cells; 2 of the 9 cells (R1 and R2) express either the UV or the blue opsin, while the remaining 6 or 7 express the long wavelength opsin. The fourth surprise in this roller coaster ride came when Briscoe realised that by expressing different combinations of the two blue opsins and UV opsin in the R1 and R2 cells of the ventral eye *L. rubidus* has increased the number of ommatidia from the three found in most butterfly eyes, to six. Briscoe suggests that this increased colour sensitivity, coupled with the early evolution of the second blue opsin gene, could have driven many lycaenid butterflies to evolve their startlingly blue wings.

10.1242/jeb.02451

Sison-Mangus, M. P., Bernard, G. D., Lampel, J. and Briscoe, A. D. (2006). Beauty in the eye of the beholder: the two blue opsins of lycaenid butterflies and the opsin gene-driven evolution of sexually dimorphic eyes. *J. Exp. Biol.* **209**, 3079-3090.

MALE SONGBIRDS MODULATE SICKNESS BEHAVIOUR



Pete Jeffs is an illustrator living in Paris

When fighting an infection, most animals mount a whole suite of physiological responses, ranging from specialist immune cells that combat the invader, to a variety of behavioural responses, known as sickness behaviour. However, activating an immune response can be an expensive exercise, so at certain times of year animals cut their costs by reducing their immune function. But do they modulate their sickness behaviour seasonally too? Noah Owen-Ashley and John Wingfield decided to test Northwestern song sparrows' sickness behaviour responses at different times of the year to see whether they modulated the behaviour seasonally (p. 3062).

Injecting male songbirds with bacterial lipopolysaccharide to simulate a bacterial infection in the winter and spring, the team monitored their aggression levels 24 hours later to see how the animals faired. During the winter, the treated males lost interest in defending their territory and even lost weight; their sickness behaviour was strong. However, during the spring when breeding occurs the treated males were every bit as feisty as the untreated males; they seemed to have lost their sickness behaviour. The spring males were modulating their sickness behaviour seasonally, probably because they had higher stress hormone levels that suppress the immune system and lower energy reserves than the over-wintering animals. Indulging in a spot of sickness behaviour is probably a luxury that breeding males can't afford.

10.1242/jeb.02453

Owen-Ashley, N. T. and Wingfield, J. C. (2006). Seasonal modulation of sickness behavior in free-living northwestern song sparrows (*Melospiza melodia morphna*). *J. Exp. Biol.* **209**, 3062-3070.

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