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

INTER-SEXUAL MIMICRY

BI-GENDER CROSS-DRESSING FOOLS RIVAL SUITORS

Cephalopods are brainy creatures capable of performing very complex tasks. Amongst cephalopods, cuttlefish are particularly remarkable because they have the extraordinary ability to display intricate changes in colour and shape, which they utilise as a form of visual communication. Their colours convey specific signals, for example during courtship, aggression or to fend off predators. Although in an ideal world communication signals amongst individuals should always be honest, deception can sometimes be fruitful; that is, if you're not caught! It is a gamble taken sometimes when the benefits that can be gained from cheating are worth the risks of suffering the consequences if the fraud is discovered.

Because of the complexity of the cuttlefish colour signals and the very fast rate at which these can change, dishonest signalling seems like a tactic from which cuttlefish could benefit – especially as mourning cuttlefish, *Sepia plangon*, populations are male biased and males compete for females, frequently interrupting each other's courtship displays. In a paper recently published in *Biology Letters*, Culum Brown and his colleagues from Macquarie University in Sydney describe how male cuttlefish deceive other males into believing they are actually females while at the same time trying to court a female; in this way, they can avoid being interrupted during their love dance. Brown and his team noticed that in some instances when a male was courting a female in the presence of another male, the side of the courting male's body facing the other male would exhibit female colouration while the side facing the female would display courtship signals. To determine the frequency and success rate of these deceptions in different types of social groups, Brown and his team observed the visual displays of mourning cuttlefish in the wild in 108 courtship groups. They took photographs of the focus male in the group – that is, the male that was performing the courtship display – and then of all other individuals within the group. In addition, they made similar observations of courtship displays in captive individuals.

The researchers observed many different types of social groups, some with more than one female and some with more than one male, but they only observed deceptive tactics in groups where there was a male courting a single female in the presence of one competitor. This tactic was employed 39% of the times within this social group and through observations on the captive cuttlefish the investigators were able to confirm that successful mating occurred when this tactic was used.

Their results suggest that cuttlefish have very high cognitive capabilities. The authors believe they are able to gauge how likely they are to get caught when using deception, as they only use this tactic when in the presence of one other male, when they are least likely to get caught. Furthermore, in instances when deception was used, the courting male would position himself between the other two individuals such that the female could only see the courtship signals while the other male could only see the female colouration. It's been said that all is fair in love and war. Deceit seems to be fair in cuttlefish and when competing for a potential mate, it is love and war all at once.

10.1242/jeb.077859

Brown, C., Garwood, P. G. and Williamson, J. E. (2012). It pays to cheat: tactical deception in a cephalopod social signalling system. *Biol. Lett.* **8**, 729-732.

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PROTECTING SPERM IN SEMINAL FLUID

Semen is a complex organic fluid that contains sperm and seminal fluid proteins (SFPs), the latter of which are produced by different secretory tissues of the male reproductive tract. In insects, hundreds of SFPs have been identified over the last few years. While little is known about their pre-mating functions in males, in females some SFPs have been shown to induce physiological and behavioural changes after mating, including changes in the willingness to re-mate, egg production rates, sperm storage, innate immunity, as well as flight and feeding behaviour. A recent study by a US team of researchers led by Reddy Palli, from the University of Kentucky, focused on identifying SFPs that are transferred from the male to the female in the red flour beetle, *Tribolium castaneum*, in the hope of identifying their molecular role in female reproduction.

To analyse SFP function, the team chose *T. castaneum* as a model, because of its fully sequenced genome and its amenability to systemic RNA interference (RNAi), a method that allows quick and efficient knock-down of gene expression. In a previous microarray study, Palli's team had identified 112 SFP genes from *T. castaneum*. However, it was unclear which SFPs are actually transferred to the females' sperm storage organ, the spermatheca. To identify SFPs that are transferred during mating, the scientists analysed protein extracts from seminal vesicles and spermathecae by mass spectrometry, as well as comparing the relative gene transcript levels of the identified proteins in both organs. In doing so, they identified 13 SFPs that were present in both seminal vesicles and spermathecae. These transferred SFPs included heat shock proteins, protease inhibitors, an angiotensin-converting enzyme (TcACE), and three further proteins that had not previously been identified in the seminal fluids of other insects. Next, they individually knocked down their expression by injecting gene-specific dsRNA into male pupae. Injected males were then mated with virgin females and the number of eggs laid was determined and compared with that of matings with males who had normal levels of SFPs.

The gene that showed the most significant effect on female reproduction was *TcACE*. Its knock-down in males caused a significant reduction in egg production by females. To exclude the possibility that the observed effect on egg production is caused by a reduced number of sperm, the team injected dsRNA into pupae and determined the sperm number in testes prepared later from 5 day old adult males. The knock-down of *TcACE* expression did not affect sperm numbers, although *TcACE* transcript levels were significantly decreased in the testes and total SFP amounts were diminished. However, sperm quality was affected, because there was a higher portion of abnormal sperm in the testes of dsRNA-injected males, suggesting that TcACE protects sperm in the seminal fluid. Finally, the scientists also examined *TcACE* gene expression in females. They demonstrated that *TcACE* is expressed not only in the male reproductive tract but also in spermatheca, suggesting that females sustain sperm protection during storage by continuing TcACE production. Speculating about TcACEs' molecular role in *T. castaneum*, the authors point out that angiotensin-converting enzymes have been detected in seminal fluids from insects to humans. As these highly conserved enzymes are known to convert hormone precursors into their active forms, TcACE may be involved in processing peptide hormones that protect sperm.

By using the powerful RNAi technique in the model beetle *T. castaneum*, Palli and his colleagues have provided convincing evidence that TcACE plays an important role in protecting the sperm in the seminal fluid during transfer and in the spermatheca during storage.

10.1242/jeb.077842

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FLIGHT CONTROL, THIS IS THE ANTENNAE

Most of us think of insect antennae as super-sensitive olfactory organs, or twitchy tactile projections. Recent findings, however, suggest a new role for insect antennae: providing feedback on the insect's orientation during flight.

The visual system and optic flow are often implicated as the major sensory input to insect flight, but over the past few years, the emerging importance of the antennae during flight has been demonstrated again and again. While insects usually hold their antennae close to their heads, flying insects will move their antennae to face forward. Damaging an insect's antennae impairs its ability to control flight speed. In moths, the antennae are also important for sensing body orientation and position, and the sensory neurons associated with the antennae are specialized for detecting body rotation. Chopping off the ends of a moth's antennae reduces its ability to steer. Despite numerous studies, how the sensory information provided by antennal deformations is incorporated into flight behavior remains a black box. Armin Hinterwith and his colleagues at the University of Washington, veterans of the study of hawkmoth flight, decided to manipulate the moth's antennae to determine the locomotor consequences of antennal deflection.

To address this question, Hinterwith and his team designed tiny, wireless muscle stimulator control boards that could be attached to a moth's abdomen. These stimulators acted on the muscles at the base of the left antenna during free flight. Muscle stimulation caused the moth's antenna to deflect backwards, replicating the air-induced deflection a moth's antennae might experience if they were perturbed during flight in nature. For instance, if a moth were forced by an external disturbance to pitch downwards, its antennae could well be pushed backwards. A disturbance causing the moth to yaw left or right, or to roll about its longitudinal axis, might cause different antennal deflections. The neurons at the base of the antenna responded to the deflection, demonstrating the moth's ability to receive mechanosensory information from the antennae.

Finally, Hinterwith and his colleagues used high-speed videos of free-flying moths to reveal any changes in flight during and after antennal deflection, yielding insight into the specific role of antennae in altering flight. As the antenna was deflected, Hinterwith found common changes in the moths' flight paths. The moths would move their abdomens, pitching the front of their bodies up. This pitch response is exactly what Hinterwith and his colleagues expected if the antennae were acting as part of a pitch-stabilizing control system. Insect body orientation, and body pitch in particular, is inherently unstable. By combining sensory information from the visual system and antennal mechanosensation, the moths can rapidly correct their flight after a perturbation.

Hinterwith and his colleagues suggest that the complex mechanosensory feedback provided by the antennae is just one system of many that is used to control flight behavior. The ability to manipulate these systems during free flight may eventually reveal how the antennae's multiple senses combine to contribute to insect behavior. But given the tremendous sensory contribution of these smelling, touching, orienting structures, the revelation may take a while.

10.1242/jeb.077826

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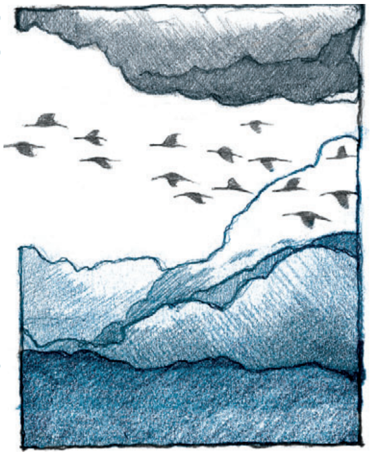
FLIGHT DYNAMICS



POWERED FLIGHT FROM PASSIVELY FLAPPING WINGS

Flags flap in the wind. What if wings also did? A recent paper from Oscan Curet, Sharon Swartz and Kenneth Breuer at Brown University shows that an aeroelastic instability, similar to flag flapping, could have provided an impetus for some gliding animals to shift to actively flapping their wings during flight.

Scientists have long debated how animals first evolved the strong wing muscles and shoulder joints necessary for flapping flight. Gliding might seem to be a natural first step on the way to fully powered flight, but gliders typically don't have the types of shoulder girdle or muscles required for flapping motions. So how could flapping evolve from gliders? Scientists believed that strengthening the wing muscles wouldn't be a sufficient starting point for evolution of active flight, as slightly stronger wing muscles still wouldn't be strong enough to power lift-off. But what if the wing muscles weren't for powering take-off, but rather were for controlling passive flapping?

Curet and his colleagues decided to examine how passive flapping motions – generated without muscle, solely due to interactions between the flexible wing and the air around it – might arise in a wing-like model, and what their effects might be on lift and drag forces. They built a simplified physical model of a wing, with flexible joints: a 'shoulder' joint that let the wing flap up and down, and a flexible flap on the trailing edge of the wing that allowed it to curve towards its trailing edge. Then they attached the wing to a load sensor that could measure forces, and put the whole setup in a wind tunnel.

At relatively low wind speeds, the wing behaved much like a classical airplane wing and remained stationary. Furthermore, like a normal wing, as they tilted and increased the angle of the leading edge, it generated more and more lift. As they increased the wind speed, though, all of a sudden the wing would start flapping. It began to oscillate up and down, though at a relatively low frequency, lower than birds or bats use when flying. Airplane engineers are quite familiar with this 'flutter' instability. However, if an airplane wing starts flapping, it often falls off, which is generally frowned upon and so, no one had considered the biological implications of fluttering wings.

When Curet measured the forces on the wing after it started fluttering and compared them to those obtained when the wing was stationary, he found a substantial increase in both lift and drag forces. As the wing flaps, a large vortex forms on the leading edge of the wing, enhancing lift, but also increasing drag. The transition to fluttering wings happened at wind speeds that would occur during typical flight speeds of flying animals. Only very slow gliders would not have to deal with the instability; others would have to pass through the transition as they changed their flight speed. But sudden changes in forces, which occur when fluttering starts or stops, tend to make it hard to control and stabilize flight.

If gliding animals want to avoid this instability, they face a choice: they can either attempt to suppress the transition (as airplane engineers do) by making the wing and shoulder stiffer or they can enhance it, by developing muscles that can flap the wing and maintain the fluttering instability below the speed at which the transition occurs passively. Both strategies would allow them to avoid the unpredictable changes in forces at flight speeds that induce the transition. In the latter option however, once muscles had evolved to control the passive flapping motion, the muscles might gradually increase in strength and this could then allow the animal to increase the flapping frequency, increasing lift even further, until fully powered flight became possible.

10.1242/jeb.077834

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