

## OUTSIDE JEB

### Different strokes for balanced birds



Hummingbirds are small, nimble fliers that must work to remain stable in the face of gusts of wind. Not much is known about how they deal with gusts that could potentially roll them upside down, rotating them along an axis running the length of their body from head to tail feathers. So, to learn more, Sridhar Ravi at the University of New South Wales, Canberra, Australia, together with an international team of researchers from the USA and Japan decided to study how hummingbirds maintain their stability when wind conditions try to roll them over. They hypothesized that the birds would remain stable in the perturbing wind gusts by flapping their wings differently on either side of the body in order to counteract the forces of the wind.

The team used four hummingbirds for flight tests in a 6 m long wind tunnel with an air speed set to  $5 \text{ m s}^{-1}$ . To control whether the birds were flying in calm conditions or turbulent air that could knock them off course, the team placed a vertical baffle in the tunnel that they could move manually in order to redirect the air flow. They then placed a syringe full of sugar water midway along the wind tunnel so that the birds would fly into the headwind in the correct location while sipping the fake nectar, ready to be disturbed when the scientists redirected the airflow from one side. Movements of the birds were tracked in 3D using white dots painted on the body and wings, and three high-speed cameras. In addition, the team surgically

implanted small wire electrodes in the muscles that flap the wings to measure their activity.

When the team looked at how the birds flew in perturbing winds, they found subtle differences between the left and right wing: one wing traced a figure of 8 while the other traced an oval shape. This was accompanied by a greater wing rotation: the front edge of the wing on the side of the body that lifted in the gust tipped down more. These subtle differences between the movements of the wings were achieved with greater activation of the flight muscles on the side of the body downwind of the gust.

Next, the team wanted to pinpoint which part of the birds' response was the most important adaptation to maintain their stability in the wind tunnel. To do this they used computer simulations to deduce how the altered movements of the wings affected the counteracting forces that the birds produced to restore their balance. By simulating different combinations of wing movements, the team concluded that the rotation of the wings was critical for the birds to remain stable.

So, Ravi and colleagues found that the subtle adaptations of wing movements are critically important for birds to remain stable. More impressively, it turns out that hummingbirds are getting two bangs for their stability buck by using the same control mechanisms that they use when manoeuvring agilely to keep them on course when caught in gusts. This new information may even inspire engineers to build more stable flying robots with improved agility.

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### Honeybees on treadmills uncover natural remedy for fungicide effects



In orchards, farmers combat harmful pathogens like fungi, which can destroy their crops, using chemical weapons such as fungicides. However, bees are often the victims of collateral damage in this perennial battle. Some fungicides affect the amount of energy organisms can produce. Ling-Hsiu Liao and colleagues from the University of Illinois at Champagne-Urbana, USA, had a hunch that if honeybees consumed fungicide, they might produce less energy in the muscles that they use for flying and their flight performance could suffer.

The team recently built a honeybee treadmill, in which the insects are tethered while flying, to investigate how a fungicide, boscalid, affects their flight. The bees wore tiny iron plates so that they could be carefully attached to a magnetic rod while flying. In addition to furnishing their treadmill with tiny televisions that tricked the bees' eyes into thinking that they are moving forwards, the researchers installed fans blowing on the bees to dupe them into thinking that they were flying freely.

To investigate the effects of the fungicide on flight, the researchers supplemented the pollen diet of a colony of honeybees with boscalid-laced sugar water. Then, they selected bees that were keen to fly outside of the hive and set the insects flying on the treadmill. The fungicide-fed bees beat their wings together at a lower

frequency than bees that did not have a fungicide-supplemented diet. As wingbeat frequency affects flight speed in other insects, it seems likely that fungicides make honeybees slower, affecting their ability to find food quickly before running out of energy.

While this might seem like grim news for honeybees, the researchers had an idea that a natural chemical in bee food could ameliorate these effects. They wondered whether quercetin, which occurs naturally in pollen, could increase the amount of available energy that bees have for flying. The researchers raised another colony of bees fed a quercetin-rich diet. They then measured the chemical that bees use to power flight – adenosine triphosphate (ATP) – in the insects' flight muscles and found that the bees that had been fed quercetin had much more ATP than those that had not received a quercetin supplement. This suggests that quercetin in bee food increases the amount of energy bees have for flying, which could help them fly faster or longer, to offset the negative effects of the fungicide.

To test this theory directly, the researchers took honeybees from another colony that had been fed on quercetin and the fungicide and put them through their treadmill test. As predicted, bees fed on the two chemicals had a wing beat frequency very similar to that of bees that had not had their diet supplemented, supporting the researchers' theory that quercetin in bee food remedies the impact of the fungicide on flight.

While quercetin seems to relieve the negative effects of fungicides on honeybee flight, other species may not be so fortunate. Liao's treadmill could hopefully help scientists understand the negative effects of pest control on other pollinators and inform future conservation efforts to protect those species.

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## Flies walk the line for serotonin



In the grand scheme of the animal kingdom, insects are often overlooked for their impressive locomotor skills. They can walk forwards, backwards and even upside down, traversing challenging environments with relative ease. But how do insects achieve this coordination? Clare Howard (Columbia University) and colleagues teach us that the insect nervous system employs a multifaceted signalling network to optimize their speed, gait and reaction timing to gracefully navigate through life.

Using the vinegar fly (*Drosophila melanogaster*), Howard and colleagues surveyed the fly nervous system to establish the sequence of signals involved in locomotion. The neural circuits responsible aggregate in the ventral nerve cord, their equivalent of the vertebrate spinal cord. The ventral nerve cord is the remote control driving the fly's three pairs of legs and it is so in tune with the fly's movements that it can even stimulate typical leg behaviours, such as walking and grooming, after a fly has been decapitated. But how does the ventral nerve cord control fly movements on such a fine scale? The authors looked to the body's built-in messaging system – the neuromodulators – to obtain the answer. Neuromodulators, such as serotonin, dopamine and noradrenaline (norepinephrine), work to transmit signals about the environment to the nervous system for processing, which are then sent back to the limbs to trigger an appropriate response.

First, the authors manipulated the fly's genetics to activate neurons associated with each of the major neuromodulators. Surprisingly, fly walking speed only shifted when the serotonergic system was activated, with no changes observed in any of the other neuromodulators. In the flies with serotonergic activation, walking remained consistently slower and straighter

than in normal flies, providing credible evidence for the role of serotonin in their agility. To confirm a direct link between serotonin and locomotion, the authors then altered the fly genes to turn off their serotonergic neurons, which caused them to walk faster and in more circuitous walking paths. Remarkably, the flies continued to walk more slowly when serotonin was activated regardless of the environment in which they were tested, with serotonin activation reducing walking speed even under varying temperatures, terrains, orientations and degrees of hunger.

Efficient movement is especially vital when predators attack. Many animals use their own unique version of 'fight or flight' at the first sign of a threat, ready to make a break for safety. Although these complex escape manoeuvres are typically attributed to higher vertebrates, such as mammals and fish, flies also display escape tactics in response to threatening stimuli, often beginning with a short freezing period that allows the fly to initiate the appropriate response. But the role of serotonin in dictating the fly's locomotor response to these threats was unknown. Howard and colleagues found that if serotonin is inhibited, flies fail to 'freeze' and think through their response, causing them to take longer to reach an appropriate walking speed when threatened. This change in behaviour when serotonin is lost could greatly increase their chance of being eaten, suggesting that serotonin acts as a locomotor hand-break and is an essential cog in the flies' threat response machinery.

Excitingly, these findings provide evidence that insects use serotonin in much the same way as fish and mammals. As serotonin dictates movement in complex vertebrates, it is likely that this mechanism developed first in simpler animals, like insects, and subsequently evolved in the more complex species in which it is observed today. Thus, serotonin helps animals from flies to cats walk the straight and narrow through life.

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## Drag lifts birds to the skies



Birds are impressive and agile fliers. To remain airborne, they must produce forward thrust and upward lift forces while overcoming gravitational and drag forces on their body and wings. Thrust drives a bird forward, while lift forces support weight and drag forces act against the wings' flapping movements, increasing the energy requirements for flight. Yet, previous studies have shown that birds orient their wings differently during take-off and landing by inclining the wings steeply as they flap, raising the question: do lift and drag fulfill different purposes during the start and end of flights? Diana Chin and David Lentink at Stanford University, USA, sought to answer this question by investigating the take-offs and landings of Pacific parrotlets to understand how the forces produced by their wings allow them to lift off and land.

However, measuring aerodynamic forces produced by a freely flying bird is no easy feat, so Chin and Lentink developed a box called an aerodynamic force platform (AFP) that measured the birds' flight forces by sensing the air displacement on its sides as the animals flew through it. Using the combination of these forces and simultaneously filmed high-speed videos of the birds' wing movements, they were able to reconstruct the magnitude and direction of the drag and lift components of the force produced by the birds during short flights from one perch to another.

The videos showed that birds steeply incline their wing beats during take-off and landing. During take-off, the birds positioned their wings to direct the drag forces upward and support almost half of their body weight. However, when coming in to land, the parrotlets flapped their wings at an angle that shifted the lift forces backward to act like a brake during the final wing beats. Increasing drag forces to aid take off is energetically

expensive, although their use of lift for braking probably saves energy when landing. Together, these findings flip the conventional understanding of the purposes of lift and drag on its head. It turns out drag might not be such a drag after all and lift can be a great brake.

It also turns out that these short parrotlet flights may show us how the ancient ancestors of the birds we know today first reached for the sky. Several theories could explain how these predecessors took their first flights; flight may have originated when they made gliding falls from trees or from running starts on the ground. Both behaviors require that would-be fliers overcome the challenge of wings that were not built to support their weight. The use of drag in modern parrotlets shows that while these wings may not have been able to produce enough lift, they could have used drag to generate the force required for take-off or to stay aloft longer. Our understanding of animal flight for both modern birds and their ancient counterparts is enhanced by considering how forces act on flapping wings.

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## Fish larvae are oxygen fans



Many fish hatch with stubby gills that have little surface area for oxygen uptake. Instead, most oxygen comes and goes across the skin over the first few weeks of development. Regardless of whether this process of gas exchange happens via the gills or the skin, it all comes down the

boundary layer, a thin coat of stagnant water that envelops the larvae as water flows around it. A thick boundary layer makes it more difficult for gases to move into (or out of) the larvae.

Fully formed gills have a boundary layer too, but the rhythmic opening and closing of the fish's mouth keeps it from getting too thick. The notion that larvae may also ventilate their skin, by swimming or fanning their pectoral fins, has intrigued physiologists for decades. However, no direct evidence linked fin movement with managing the oxygen boundary layer, oxygen uptake or metabolism in larvae. In a new paper, Alex Zimmer, Milica Mandic and colleagues from the University of Ottawa, Canada, tested the idea that pectoral fin movement plays a role in oxygen uptake in larval zebrafish and rainbow trout.

First, the researchers measured how quickly zebrafish larvae flapped their gills and fins as the oxygen availability was reduced to produce hypoxic conditions. Just like adult fish, the larvae of all ages (4–21 days post-hatching, dph) ventilated their gills faster during hypoxia. As the larvae developed, their fin-beating behaviour changed: young (4 dph) larvae furiously fanned their fins when the oxygen levels were low, whereas older (15 dph) larvae waved their fins less urgently. The oldest larvae (21 dph) barely beat their fins at all. Most intriguingly, the larvae ceased beating their fins frantically just as the gills took over as the main site of gas exchange.

Intrigued, the team measured oxygen levels along the skin surface of trout larvae with a precise oxygen sensor. They found that regions near the pectoral fins, like the front of the yolk sac, were more oxygen rich than areas towards the tail. And when the scientists anaesthetised the larvae to briefly halt fin and gill ventilation, the oxygen concentration in the water near the fins decreased by as much as 50%, demonstrating that fin beating was responsible for the high oxygen levels in these body regions. What's interesting is that surgically removing the pectoral fins, but leaving the gills intact, had comparable effects on the distribution of oxygen along the bodies of the larvae to complete anaesthesia. Pectoral fins have a significant role in stirring up the oxygen boundary layer and maintaining high oxygen levels at the skin surface.

To definitively link fin beating with oxygen uptake and metabolism, the team compared whole-animal oxygen consumption rates in intact and fin-clipped larvae exposed to hypoxia. The oxygen consumption rates were markedly lower in the fin-clipped trout larvae, supporting the notion that fanning supports oxygen uptake in hypoxia. Interestingly, fin-clipped zebrafish larvae maintained their oxygen consumption rates despite low water oxygen levels, hinting that the higher

activity levels or greater hypoxia tolerance of zebrafish larvae may mitigate the effects of pectoral fin removal on oxygen uptake.

Just like adult gills, the skin of larval fish is only as good as its (lack of) boundary layer. These observations are the first direct evidence for the respiratory role of pectoral fins, which facilitate oxygen uptake by dissipating the boundary layer in at least one species. For young larvae, oxygen uptake is more than skin deep.

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