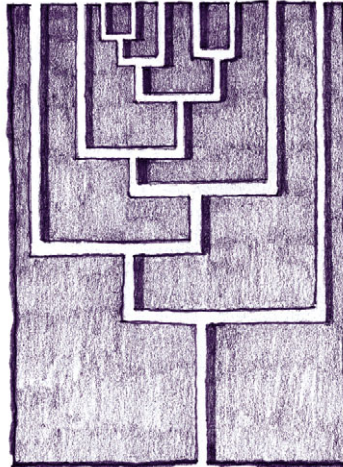


Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

EVOLUTION



GETTING A JUMP ON THE COMPETITION

Imagine the scene; the camera pans from a Petri dish of wriggling larvae to a sexy blonde scientist who is peering at the animals through a microscope. She looks up, terror-stricken, and says into the camera 'My God, they're evolving!' While TV and movie scenes like this make most biologists chuckle, Australian conservation biologists who are working to contain an invasion of exotic cane toads may not find them funny. This is because a recent paper by Phillips and colleagues has demonstrated that introduced cane toads in Australia have evolved in the 70 years since their arrival in ways that are accelerating their conquest of the continent.

Cane toads (*Bufo marinus*) are a South American species that was introduced to Queensland in 1935 as a strategy for controlling insect pests in sugar cane fields. Like many of the stories involving intentional introduction of species by humans, the cane toad story is a textbook illustration of how ecological short-sightedness can have disastrous long-term consequences. Not only did the cane toads fail to control the cane beetles they were recruited to keep in check, but they are currently eating their way across Australia, outcompeting native frog species (Australia has no endemic toads), and pushing many endangered species to the brink of extinction. The toads continue to expand their range at an alarming rate in spite of intense efforts to control their spread to the rest of the country.

To investigate toad dispersal, the authors of this paper tracked cane toad movements using radio telemetry and found that they can move up to 1.8 km per night during the rainy season. Cane toads are one of the world's largest anuran species, but this

kind of movement is truly remarkable among anurans, and it got the authors wondering if natural selection has resulted in a population of toads that is better adapted at dispersal. Their reasoning was that the introduction of cane toads to Australia has resulted in a unique opportunity for the toads, where prey is plentiful and experienced predators are rare. Because the proliferation of toads in a given area will eventually diminish prey availability, those toads that can continuously move into virgin territory will be richly rewarded for their efforts in terms of reproductive output.

It is known that leg length in anurans correlates positively with hopping speed, so the authors first tested whether this was true in cane toads, and indeed it was – longer legged toads covered more ground per night. To test whether long-legged toads reach virgin territory first, the researchers set up shop just in front of the invasion front and measured leg lengths of the toads as they swept past. Sure enough, the toads with the longest legs tended to arrive first, and the ones with the shortest legs tended to arrive last, suggesting that long-legged individuals enjoy the benefits of dispersal into new territory more than their lagging short-legged conspecifics. While these results strongly suggest positive selection for long-leggedness in the toads, it doesn't prove that the population is actually evolving. For this, the authors looked to historical records and preserved specimens, and concluded that leg length has increased significantly in cane toads since their arrival. The authors suggest that increased leg size has resulted in a population of cane toads that is better adapted for dispersal than their founding ancestors. This is consistent with historical records that show a fivefold increase in the rate of range expansion by the toads since they were introduced, from 10 km per year originally to over 50 km per year now. This study sends a sobering message to conservation biologists that exotic introductions are best dealt with swiftly, before the founder population has a chance to evolve into an even more formidable opponent.

10.1242/jeb.02283

Phillips, B. L., Brown, G. P., Webb, J. K. and Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature* **439**, 803.

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COLOUR VISION



COLOURFUL DAYS, COLOURLESS NIGHTS

Animals use colour vision for a variety of behaviours including finding food or identifying a mate. Colour vision in primates, for instance, is thought to have evolved because it improved the discrimination of fruit from surrounding foliage. Although the details of visual neural circuits differ between species, the underlying principles of colour vision are similar across most species; they require at least two sets of photoreceptors, each with different spectral sensitivities. Most mammals possess two sets of cone photoreceptors, tuned to absorb light from different parts of the visible spectrum, one usually tuned for short wavelength sensitivity and the other a long wavelength sensitive opsin. In early mammals, the short wave sensitive (SWS) opsin is thought to have been tuned to ultraviolet wavelengths, but in ground squirrels indirect evidence suggested that the SWS opsin's sensitivity had shifted to the violet region of the spectrum. Livia Carvalho and her colleagues at University College London set out to find the molecular changes that occurred in the tree squirrel opsin to shift its peak sensitivity from ultraviolet to violet light.

Obtaining the complete coding sequence of the grey squirrel (*Sciurus carolinensis*) SWS opsin, the team expressed the *opsin* gene before combining the protein with the light sensitive chromophore, 11-*cis*-retinal, to reconstruct the functional photopigment. Having purified the opsin the team measured the photopigment's absorption spectrum and found that the grey squirrel's SWS opsin did indeed have a peak absorption in the violet range of the light spectrum. When they compared the grey squirrel SWS opsin amino acid sequence with that of a typical ultraviolet sensitive pigment from the mouse, they noticed that at one position a phenylalanine (Phe) had

been substituted with a tyrosine (Tyr). To determine whether this substitution is sufficient to produce the shift in the absorption spectrum, they constructed a grey squirrel opsin sequence in which the Tyr was replaced by Phe. When the absorption spectrum of this opsin was measured it was shifted back towards the ultraviolet, confirming that the Phe to Tyr substitution was responsible for the spectral shift.

Next, to see whether similar changes had occurred in other closely related species, Carvalho and her colleagues obtained partial sequences for SWS opsins from two flying squirrels: the Siberian flying squirrel (*Pteromys volans*) and Northern flying squirrel (*Glaucomys sabrinus*). The SWS sequences from both species contained a Tyr at the same position as in the Tyr in the grey squirrel's opsin, suggesting that the flying squirrels' peak sensitivities had also been shifted from the UV to violet light. However, both flying squirrels' *opsin* genes contained deletions that prevented the formation of functional opsins. This means both of these species lack colour vision, but this may not be a bad thing as flying squirrels are nocturnal. Many nocturnal animals lack colour vision because they must use all the available photons to get sufficient spatial and temporal resolution; by requiring multiple separate neural channels, colour vision may reduce spatial and temporal resolution.

Remarkably, many other mammals that possess a violet-sensitive opsin, such as cows, pigs and wallabies, also show a similar Tyr substitution for a Phe at the same location in the *opsin* gene, suggesting that the violet opsin has arisen many times during the course of evolution through convergent evolution. Loss of the SWS opsin has also occurred numerous times in nocturnal and marine mammals. Convergent changes in molecular structure have clearly played an important role in the evolution of the mammalian visual system.

10.1242/jeb.02284

Carvalho, L. dos S., Cowing, J. A., Wilkie, S. E., Bowmaker, J. K. and Hunt, D. M. (2006). Shortwave visual sensitivity in tree and flying squirrels reflects changes in lifestyle. *Current Biology* 16, R81-R83.

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PROTEIN FUNCTION



CHEW ON THIS

The Gila Monster (*Heloderma suspectum*) is one of only two lizard species believed to be venomous. This lizard produces a peptide, exendin-4, exclusively in the salivary glands, that is thought to be used for prey capture or defense. However, a recent study where the Gila Monster's natural prey and predators were injected with exendin-4 found that the protein did not impair the victims at all. Another recent study found that plasma levels of exendin-4 in Gila Monsters increased dramatically following feeding. Thus, rather than being a venom component, exendin-4 may have a physiological role in digestion and absorption-related events. Carolyn Christel and Dale DeNardo of Arizona State University were interested in investigating what this role was, and since no one had previously studied this interesting problem, the team decided to start at the very beginning. They wanted to know the mechanism controlling the protein's release during feeding.

They surmised that the postprandial elevation in circulating exendin-4 levels could result from secretory stimuli associated with prey detection, prey capture and/or prey digestion/absorption. However, given that exendin-4 originates in the salivary glands, the team hypothesized that mechanical events associated with prey capture (i.e. biting and chewing) would provide the greatest stimulus for exendin-4 release. To test their hypothesis, the team measured Gila Monster plasma exendin-4 levels before and at 15 min, 45 min and 24 h following one of six treatments designed to test the effects of different feeding actions and food types on exendin-4 release. The treatments consisted of: (1) lizards fed gelatinous egg mixture, (2) lizards fed intact juvenile rat, (3) gastric intubation of anaesthetised lizards with gelatinous egg, (4) stimulation of unfed

lizards with egg scent, (5) unfed lizards, and (6) lizards that bit, but were not fed.

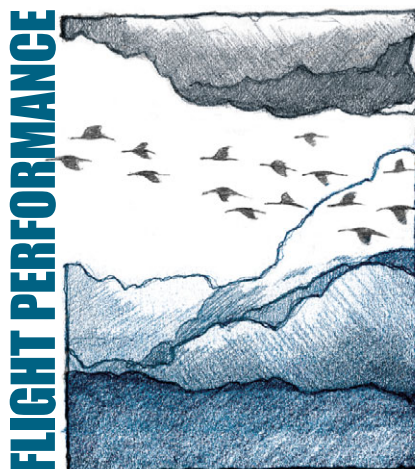
The team found that plasma exendin-4 levels increased significantly in lizards that did a lot of biting, but not in other treatment groups. In the Gila Monsters that were fed juvenile rat or stimulated to bite without eating, plasma exendin-4 levels were significantly higher at the 15 min and 45 min measurement times, but by the 24th hour, the exendin-4 levels had returned to control levels. Christel and DeNardo argue that these results suggest that exendin-4 is released from salivary glands in response to mechanical stimulation (i.e. chewing movements) and not the detection of food by smell, taste or gut distention.

Just how exendin-4 enters the blood of the Gila Monster once released from the salivary glands remains a mystery. This is an intriguing question because salivary glands are not known to have an endocrine function in vertebrates, and proteins in general have limited ability to cross cell membranes. Likewise, the physiological consequences of elevated plasma exendin-4 in the Gila Monster remain to be discovered. However, Christel and DeNardo expect that exendin-4 has an important role in digestion and food absorption following a meal. The team explains that when injected into mammalian species, exendin-4 results in a prolonged decrease of plasma glucose levels – a phenomenon that makes exendin-4 attractive as a potential therapeutic treatment in diabetes. Since Gila Monsters are binge feeders that consume very large meals at infrequent intervals, Christel and DeNardo predict that exendin-4 plays an important role in the metabolism of foods that require lengthy pre- or post-absorption processing.

10.1242/jeb.02285

Christel, C. M. and DeNardo, D. F. (2006). Release of exendin-4 is controlled by mechanical action in Gila Monsters, *Heloderma suspectum*. *Comp. Biochem. Physiol.* **143A**, 85-88.

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HIGH-FLYING HUMMINGBIRDS BURST TO BEAT COMPETITORS

Locomotor behaviour and performance has long been assumed to be the key factor in determining the outcome of competition between animals for resources. However, empirical data to support this hypothesis has proved challenging to collect. In a recent publication in *The American Naturalist*, Douglas Altshuler sought to experimentally test the hypothesis that flight performance influences both territoriality, and success when competing for niches, by comparing the flight behaviour of several hummingbird species over a range of altitudes.

Although simple measurements of wing disc loading in flying birds had previously been used to approximate the bird's flight performance, Altshuler instead used more complex aerodynamic theory to calculate more realistically the costs of flight. Altshuler also filmed the birds at different altitudes in the wild, and under low-pressure conditions in the lab, to identify their flight kinematics. Finally, Altshuler calculated the power requirements for flight, particularly in relation to maximal or 'burst' power, as well as recording features of territorial behaviour during field observations.

Travelling to the Andes and Colorado, Altshuler studied hummingbird flight in three experiments. First, he compared territorial behaviour of hummingbird species in the mountains from 400–3860 m and found that wing disc loading had no effect on territoriality, but burst power did. Territorial hummingbird species had higher burst aerodynamic power outputs than non-territorial species. Second, he studied two species of *Selasphorus* hummingbirds competing for access to nectar at 1875 and

2900 m. At the lower altitude, *S. rufus* males appeared to have a competitive advantage over *S. platycercus* males, but the roles were reversed as altitude increased. *S. rufus* had relatively lower burst power output and Altshuler found important difference in hovering kinematics between the two species of hummingbirds. Altshuler's measurements showed that *S. rufus* males used higher stroke angles when hovering and this constrained their ability to modulate aerodynamic force production when compared with *S. platycercus*. Tempting the hummingbirds with high-quality feeders (sucrose solutions) revealed that not only did altitude affect the way in which the hummingbirds selected feeding sites, but that the density of competitors for feeders also effected the bird's choice. The final component of the study involved laboratory studies of flight performance under high and low pressure conditions. Both species increased power output to compensate for lower air density.

Altshuler's study has provided several intriguing insights into both the mechanical requirements for flight of territorial birds and the influence of environment on competitive interactions. The ability to produce bursts of power appears to be a pre-requisite for successful competitive outcomes at higher altitudes; however, at lower altitudes other flight parameters (such as manoeuvrability) can improve a hummingbird's advantage. Clearly, physiological and aerodynamic limitations become more important as oxygen availability and air density decline with increasing altitude. This study has shown how a bird's flight performance, and a bird's ability to modulate it, can affect its ability to compete with other species for resources.

10.1242/jeb.02281

Altshuler, D. L. (2006). Flight performance and competitive displacement of hummingbirds across elevational gradients. *The American Naturalist* **167**, 216-229.

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MEMORY



LEARNING TO FLY

Perhaps surprisingly, learning is not a uniquely human trait; not only do very simple animals learn, but most of what we know about the mechanisms of learning comes from studies of organisms like the sea hare (*Aplysia*) or the fruit fly (*Drosophila*). In both organisms, the cyclic AMP signalling pathway has been implicated; and in *Drosophila*, classic memory genes impact directly on the cAMP pathway: *dunce* encodes a cAMP-phosphodiesterase, and *rutabaga* an adenylate cyclase. For olfactory learning, in which flies are trained to associate a particular odour with a mild electric shock (and so avoid it), the memory trace has been localised to a tiny area in the middle of the brain, the ‘mushroom body’. In this paper, Gang Liu and colleagues trained flies to avoid certain shapes in their visual fields. They showed that a different area of the brain, the ‘fanlike body’ is involved in shape recognition, and thus that there is no single, generic memory centre in the flies’ brains.

The technology to accomplish this experiment is remarkable: each fly was glued to a thin rod, attached to strain gauges that monitored the insect’s flight. The fly was then placed in a panoramic flight simulator, shown various simple shapes, and was conditioned to fly so that they kept certain shapes out of the middle of their visual field in order to avoid an aversive stimulus (heat). Previous experiments showed that flies can recognise separately at least five different parameters; size, colour, elevation in the panorama, vertical compactness and contour orientation.

To test which region of the brain was responsible for shape memory the team designed transgenic flies where they could inhibit memory formation in specific regions of the brain by expressing tetanus toxin (CnTE), a specific inhibitor of synaptobrevin (an essential synaptic protein), before conditioning the insects to avoid certain stimuli and testing their recall. By blocking memory formation in specific locations in the intact brain, the team found that the mushroom body had nothing to do with this learning task; instead the largest region of the central complex, the ‘fanlike body’ needed to be working for memory formation.

Of course, the presence of tetanus toxin might have induced some developmental deficit, rather than directly impacting on learning. So the authors used a new *Drosophila* trick. Gal80^{ts} is a protein that binds to GAL4 and inhibits it, but releases GAL4 at higher temperatures (>30°C), allowing the expression of genes controlled by GAL4. By constructing mutant flies expressing the tetanus toxin

under the control of GAL4, but in the presence of the Gal80^{ts} inhibitor, the team were able to prevent expression of the gene at normal temperatures, confirming that presence of the gene did not affect the flies’ memory formation. But as soon as the temperature rose the flies began expressing tetanus toxin and showed exactly the same learning deficits, so tetanus toxin was impacting on the learning process itself.

The authors then repeated the experiments with mutant flies lacking *rutabaga* (adenylate cyclase) that can fly and navigate, but cannot learn. By adding back normal *rutabaga* function, they showed that cAMP signalling is indeed required in the fanlike body for learning to take place.

As a final flourish, the authors were able to show that two different visual discriminations (an elevation of the whole panorama cf. orientation of the training shapes) could be associated with two distinct subsets of cells within the fanlike body. The results thus showed that while cAMP is a general property of learning, different regions of the brain are responsible for different memory modalities.

10.1242/jeb.02282

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