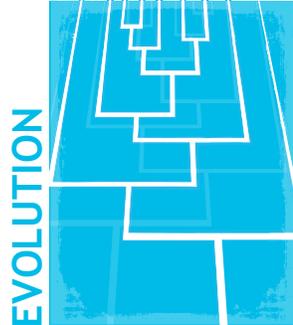


OUTSIDE JEB

A soaring success: repeated evolution of island flightlessness



Within the Seychelles archipelago of the Indian Ocean is found the world's second-largest pristine coral atoll: Aldabra. Because of its geographical isolation and the difficulties accessing it, Aldabra has long been protected from human interference. Given this unique circumstance, animal populations on Aldabra have thrived, constrained solely by nature. Although Aldabra is over 400,000 years old, the islands were flooded most recently around 136,000 years ago, wiping out most terrestrial life. Nevertheless, Aldabra was swiftly recolonized by animals once the waters receded, giving credence to Dr Ian Malcolm, in *Jurassic Park*, who memorably said, 'Life, uh, finds a way'. For scientists interested in natural history, the Aldabra atoll provides a wealth of well-preserved animal fossils, spanning the period from before to after the last inundation. Paleobiologists Julian Hume, from the Natural History Museum at Tring, UK, and David Martill, from the University of Portsmouth, UK, took advantage of this exceptional fossil record to show that flightlessness has evolved twice in the same bird species on two separate occasions on Aldabra.

The coral atoll is home to the only living flightless island bird species in the Indian Ocean, the Aldabra rail (*Dryolimnas aldabranus*), which lost its ability to fly thanks to the absence of predators allowing it to roam freely on the ground. The Aldabra rail is descended from the living white-throated rail (*D. cuvieri*), a

migratory species found widely throughout the south-western Indian Ocean that settled on the atoll and lost its ability to fly after the atoll reemerged from the sea. Interestingly, the fossil record shows that there was another flightless species of rail on Aldabra before the flood. Curious about the origins of this extinct flightless species, Hume and Martill hypothesized that it also evolved from the white-throated rail, which is notorious for colonizing isolated islands, and then quickly evolving flightlessness.

The researchers analyzed specimens of all three *Dryolimnas* species, and found remarkable similarities between the bones of the migratory white-throated rail and the extinct and modern flightless Aldabra rails. These three species share nearly identical structures at the bottom of their legs (where the foot bones meet), yet the flightless rails have much thicker leg bones that are better suited to running on land, by enhancing balance. Conversely, the researchers found that the long and slender shape of the white-throated rail's wing bones match its airborne lifestyle, whereas the short and thick wing bones of both the extinct and Aldabra flightless rails make them unfit for flight. The authors also reconstructed a timeline of the white-throated rail's evolutionary history on Aldabra – using specimens of all three species – to show a rapid transition from bones useful for flight to ones adapted to living on the ground. These findings not only show that the white-throated rail successfully colonized Aldabra twice but also that it evolved flightlessness at two distinct periods, essentially giving rise to the exact same bird.

The discovery by Hume and Martill adds an intriguing chapter to our understanding of evolutionary history, by providing the first example of recurring flightlessness in the same species of bird, and they join an extremely short list of rare examples of iterative (repeated) evolution. Furthermore, although this study illustrates the devastating effects of a sea-level rise, it also shows the resilience of nature and gives hope for how species will recover once human interference is reduced. Given sufficient time, animals

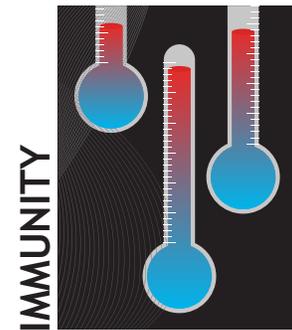
adapt to and flourish in their environments.

doi:10.1242/jeb.192898

Hume, J. P. and Martill, D. (2019). Repeated evolution of flightlessness in *Dryolimnas* rails (Aves: Rallidae) after extinction and recolonization on Aldabra. *Zool. J. Linn. Soc.* doi:10.1093/zoolinnean/zlz018

Ilan Ruhr (0000-0001-9243-7055)
The University of Manchester
ilan.ruhr@manchester.ac.uk

Sit tight: nest temperature affects infection resistance of chicks



The incubation of eggs is a critical stage in the life of a bird. Although the eggs need the warmth of their parent's body to develop, most birds need to leave their eggs for brief periods to forage for food. When the adults are gone, the egg's temperature will fall and might even reach the temperature of the surroundings if the parents don't return soon. And being exposed to the cold is no fun for the embryos; studies have found that suboptimal incubation temperatures can lead to fewer chicks hatching and lower survival rates. However, most studies on this topic have investigated the effects of a stable incubation temperature, which is seldom experienced by the embryos under natural conditions; little is known about the impact of the parent's periodic absences on their embryos. So, Benjamin Burrows and colleagues from Trent University in Canada set out to test the influence of a variable incubation temperature on the life

performance and immune function of Japanese quails.

The team incubated quail eggs at temperatures that fluctuated around an average temperature of 36°C over a continuously repeated 3 h cycle. This was achieved by switching off the incubator – which was set to 37.5°C – for less than an hour, allowing the incubation temperature to drop to 28°C, before the incubator was switched back on again to warm up. They then sampled blood from the chicks at three time points – 5 days after hatching, when the birds reached 20 days of age and after reaching adulthood – and tested the ability of the chicks' blood to kill *Escherichia coli*, a common bacterium that can cause diarrhea. The researchers then compared the immune function of the chicks that had been warmed and chilled alternately with that of chicks that were raised at a constant low incubation temperature of 36°C or at a constant 37.5°C, which is commonly used as incubation temperature in quail farms.

When the researchers compared how the immune system of the three groups of chicks developed, they found that the birds' ability to fight the *E.coli* infection increased as the chicks grew and initially they could see no clear difference across the groups; all of the chicks had similarly strong immune systems at the age of 20 days. However, when they focused on the long-term effects that the incubation temperature had on adult birds, they saw that the quails that had been exposed to the cycling temperature as embryos had the least ability to fight infections. Their immune function was markedly lower than that of the quails that were raised at constant 37.5°C, while the immune function of embryos incubated at a constant 36°C was only slightly worse than that of birds raised at the optimal temperature.

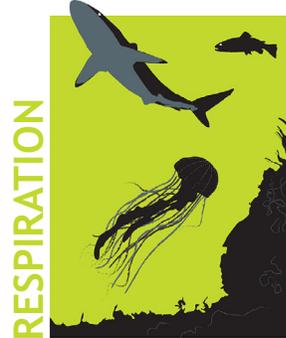
Although it is not yet understood how variation of the incubation temperature causes a decline in immune function, it is clear that incubation conditions can have a large effect on the chick's immune system and will probably affect the survival of the adult birds. The study emphasizes the importance of environmental conditions, such as food availability or predator presence, which influence the amount of time that parents spend on the nest, with implications for the life performance of their offspring as adults.

doi:10.1242/jeb.192872

Burrows, B., Ben-Ezra, N. and Burness, G. (2019). Exposure of avian embryos to cycling incubation temperatures reduces adult bactericidal ability. *Physiol. Biochem. Zool.* **92**, 286-292. doi:10.1086/702765

Julia Nowack (0000-0002-4512-5160)
Liverpool John Moores University
 J.Nowack@ljmu.ac.uk

Deep breathing in tired trout



How do animals know when to take a breath? Whether the animal is awake or asleep, and during exercise or at rest, the respiratory system carefully regulates breathing patterns to maintain oxygen uptake without the need for conscious thought. Scientists have long known that specialized cells continually monitor the pH and levels of oxygen and carbon dioxide in the blood and these cells send neural impulses to stimulate breathing if these parameters move away from normal values. More recently, experiments in rats and mice have found that the common organic compound lactate, produced by animals when not enough oxygen is available, also stimulates breathing. However, it is unknown whether this mechanism of respiratory control is unique to mammals or a trait shared by all vertebrates.

A new study, led by Mikkel Thomsen at Aarhus University, Denmark, tested the hypothesis that respiration in fishes is controlled by the amount of lactate in the blood just like it is in mammals. The authors performed careful surgeries on rainbow trout (*Oncorhynchus mykiss*), inserting a tiny tube into the bloodstream so that they could inject lactate to directly manipulate levels in the blood. They also attached an electrical probe to the bony plate that covers the gills, allowing them to measure the amplitude and frequency of the fish's breathing movements.

Just as the authors hypothesized, addition of lactate to the bloodstream stimulated trout to take larger, deeper breaths, but the frequency of breathing did not change. To see whether the fish could respond to the precise amount of lactate in the blood, the authors added different quantities through the plastic tubing and found that the more lactate they added, the deeper the breaths of the fish. Thus, lactate seems capable of transmitting detailed information about the oxygen requirements of the fish.

Next, the authors asked how trout sense changes in circulating lactate levels. Specialized cells in the gills of other fishes are known to sense oxygen levels in the blood and signal for increased breathing, so perhaps these could also sense lactate. First, Thomsen and his colleagues painstakingly removed the gills that contain most of these sensory cells and, as predicted, the breathing of these fish hardly increased when they were given lactate injections. Next, the authors injected intact fish with a variety of drugs that are known to target the gill sensory cells. In the presence of these drugs, lactate injections did not stimulate breathing, strongly indicating that the lactate signal is received by sensory cells in the trout's gills. Finally, the researchers wanted to know whether the lactate sensors in trout shared an evolutionary history with those already discovered in mammals, or whether the fish used a different mechanism to keep tabs on blood lactate. Searching through the genetic code of the trout, the authors found a gene that looked remarkably like the one that codes for the lactate sensor in mammals. Thus, it seems that all vertebrates, whether furry or fishy, may share a signalling system that uses lactate to stimulate breathing.

Of course, many more animals should be investigated before we can conclude that lactate is a universal respiratory stimulant, but the benefits of this simple signalling system are clear. When oxygen is limited, animals take an energetic loan in the form of lactate. Then, to pay off this 'oxygen debt', lactate acts as its very own debt collector, stimulating deeper breathing and hence increased oxygen uptake. No middlemen required.

doi:10.1242/jeb.192906

Thomsen, M. T., Lefevre, S., Nilsson, G. E., Wang, T. and Bayley, M. (2019). Effects of lactate ions on the cardiorespiratory system in rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol. Regul.*

Integr. Comp. Physiol. **316**, R607-R620. doi:10.1152/ajpregu.00395.2018

Andy Turko (0000-0002-6330-5798)
University of Windsor and McMaster University
 ajturko@gmail.com

Pesticides put the bumble back into bumblebees



There has been a lot of buzz surrounding the risks of pesticide pollution to bees over the past decade, with many studies reporting that pesticide-contaminated pollen and nectar can have both lethal and non-lethal consequences for important pollinating insects. While most of the research in this area has focused on honeybees, the effects of pesticides on our vulnerable wild bees are commonly overlooked. Buff-tailed bumblebees (*Bombus terrestris*) are one such insect, responsible for pollinating a range of fruit and vegetable crops, as well as countless wild plant species. Despite their importance in providing ecosystem services to farmers and orchardists, bumblebees are under serious threat from agricultural practices, and a recent study from Imperial College London, UK, has revealed that pesticides are affecting these marvellous minibeasts in more subtle ways than previously understood.

As flight performance is such a crucial element to the survival and reproduction of bumblebees, lead author Daniel Kenna and his team were interested in investigating how agricultural pesticides interfere with the flight dynamics and endurance of wild bumblebees. To keep their experimental study as realistic as possible, the researchers chose to use the neonicotinoid pesticide imidacloprid because of its widespread use in farming around the world and only applied doses similar to those that have been found previously in the pollen and nectar stores of wild bees. Prior to the experimental flights, the team fed the bees either on imidacloprid-treated sucrose or untreated sucrose, and then assessed the insects' flight performance using a circular flight mill comprising a revolving brass wire that ended in a small face-down magnet. The team then attached small metal tags to the backs of the bees which allowed them to fly continuously while remaining anchored to the mill. With this simple setup, the researchers were able to measure the number and speed of circuits that the bees performed. As bumblebees do not fly continuously while foraging in the wild, the researchers allowed each bee a maximum of five brief rest breaks before ending the flight and, after 60 minutes, any bees still flying were brought to a stop.

Interestingly, the team discovered that the average circuit speed and average maximum speed achieved by the pesticide-treated bees was actually much higher than those of the control bees. The researchers explain that this hyperactivity is not entirely unexpected as neonicotinoids stimulate neurons in a similar way to nicotine, which could explain this burst of speed. However, in a plot twist torn straight from the pages of 'The tortoise and the

hare', the researchers found that this frantic frenzy came at a big cost in the long run. The pesticide-treated bees covered much shorter distances (0.7 km) than the control bees (1.8 km) and the same was also true of flight duration, with flights of pesticide-treated bees (~14 min) lasting nowhere near as long as control bee flights (~48 min). In fact, while not a single pesticide-treated bee was able to fly for the full 60 minutes, 65% of the bees that had not received a neonicotinoid shot achieved this feat, effectively demonstrating the severe hampering of flight endurance brought about by exposure to the pernicious pesticide.

As well as underlining the serious consequences that a reduction in flight range and endurance can mean for a bumblebee's ability to forage and pollinate crops, the authors stress the importance of studying non-honeybee species, such as bumblebees, as one previous study found that honeybees actually increased flight endurance when treated with neonicotinoids, emphasising that what may be true for one pollinator species may not be true for all. Finally, by demonstrating how easily these patient pollinators can become doomed drag-racers when exposed to realistic neonicotinoid doses, this study adds to the growing body of evidence that pesticide pollution can be a real buzzkill.

doi:10.1242/jeb.192880

Kenna, D., Cooley, H., Pretelli, I., Ramos Rodrigues, A., Gill, S. D. and Gill, R. J. (2019). Pesticide exposure affects flight dynamics and reduces flight endurance in bumblebees. *Ecol. Evol.* **9**, 5637-5650. doi:10.1002/ece3.5143

Alex Evans (0000-0002-5655-012X)
University of Leeds
 alexevans91@gmail.com