

## OUTSIDE JEB

## 'CRISPR-ed' mosquitos, anyone?



Zika, malaria, dengue, chikungunya: all of these diseases are transmitted by a simple mosquito bite. Pesticides have always been our champions in controlling insect populations to limit the spread of these diseases. However, they may indiscriminately target other populations, and these effects are becoming more apparent. Ongoing public concern about pesticide use, along with the ability of insects to develop resistance, has prompted the search for alternatives.

Recent developments in molecular biology have made it possible to use the pest's genome against itself, by artificially inducing mutations in specific genes that weaken the population. Endonucleases play an essential role in this genome manipulation. These enzymes drive the cleavage of specific DNA sequences, thus disrupting gene expression and allowing researchers to insert new DNA coding for traits that weaken the population. New developments in the application of the CRISPR-Cas9 endonuclease system have simplified the process of cutting DNA sequences to inactivate or mutate genes. This approach allows researchers to convert an embryo that has two identical copies of a gene into an embryo that has a normal copy of the gene and a second, mutated copy that can be passed on to subsequent generations.

Andrew Hammond and colleagues decided to use this approach in a bid to

control the population of *Anopheles gambiae*, the mosquito that transmits malaria, by creating a mutation in a gene essential for fertility. Females with two mutated genes will be sterile, while those with one copy of the mutated gene will be fertile. Usually, this type of mutation would be removed from the population, but the authors used a novel technique called 'gene drive', which ensures that the DNA alteration persists, allowing the mutation to be passed to future generations. This novel method of pest control would yield sterile mosquitos in every generation, therefore decreasing the overall number of individuals in the population.

Identifying three genes that result in reduced fertility – two that may play roles in gamete and embryo development, and a third with a possible role in mosquito larval molting – the team was able to mutate all three using the CRISPR-Cas9 system and verify that the mutations could be passed on to the insects' offspring with an 87.3–99.3% success rate. This confirmed that gene disruptions driven by the CRISPR-Cas9 system can be propagated in the population, thus allowing DNA alterations to occur in multiple mosquito generations. Using laboratory experiments and a model to predict whether the mutations would spread if released into the population, the team showed that a mutation in the third gene would spread in the wild mosquito population and reduce their numbers, while the mutations in the other two genes would disappear over time.

The study by Hammond and colleagues is one of a few to break ground in a new era of mosquito population control. They have shown that the CRISPR-Cas9 system effectively disrupts gene expression associated with fertility and have identified a potential target gene for controlling the *A. gambiae* population. With no release of chemicals into the environment, no effects on non-target populations and a smaller chance of resistance developing, genome editing in the mosquito population could prove to be a beacon of hope for a malaria-free future.

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## Big trouble for little bats in humid caves



Small bats hibernating in humid areas of North America are in big trouble as a result of deadly white-nose syndrome. This fungal infection, caused by cold-growing fungus *Pseudogymnoascus destructans*, is now one of the fastest spreading wildlife diseases. Researchers have acted quickly to understand this disease and have already identified that the fungus infects the skin of bats during cold-weather hibernation. The infection causes bats to arouse from hibernation more frequently than uninfected bats. These metabolically costly arousals make bats consume their over-wintering energy stores more rapidly, causing them to die from emaciation and starvation. The true puzzle is that white-nose syndrome does not affect all bats equally. Certain bat species in North America are suffering high death rates, while others only experience mild or no mortality, and bat species from Europe appear to survive hibernation even with the infection.

*Pseudogymnoascus destructans* has already killed over a million bats in North America since its accidental introduction

in 2007, and researchers are struggling to identify what traits of the fungus (the pathogen), the affected bat species (the hosts) and the hibernaculum (the environment) create the ‘perfect storm’ of circumstances to increase bat mortality. David Hayman of the Hopkirk Research Institute at Massey University, New Zealand, and his colleagues wanted to identify the factors that leave some species unaffected, while others quickly perish.

The team assembled information about the habitat and hibernation conditions of two North American bat species (the highly impacted little brown bat and the less impacted big brown bat) and two European bat species of similar sizes (the serotine bat and the greater mouse-eared bat), which they then incorporated into a computational model to predict fungal growth over a range of bat body temperatures in environments with different humidities. They added this information to models that calculate bat metabolism at various hibernating temperatures to ascertain how the bats consume their energy reserves. They were then able to predict bat survival over a range of hibernation durations and habitats that they are known to occupy.

The models predicted that bats with small body sizes – similar to those of the little brown bat – that hibernate in more humid and warm caves would succumb to the disease faster and more often than larger bat species from drier caves. They revealed that the fungus grows fastest in humid conditions and that smaller bats, which have fewer energy reserves to waste on frequent arousals, will be most affected by the disease. In fact, the models were impressively accurate, reproducing the pattern of mortality seen in North America (high to low mortality) and Europe (low to no mortality).

Together, Hayman and his colleagues have solved an important part of the white-nose syndrome puzzle, showing that the humidity of the hibernating environment is an important determinant of disease progression. They conclude that their results present ‘a bleak picture’ for small-bodied North American bats. More broadly, they show that understanding interactions between the disease triad – the pathogen, the host and

the environment – will help us to quickly understand other deadly diseases that are spreading rapidly.

10.1242/jeb.130104

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## How a fern designs a catapult



The fastest known catapult in the plant kingdom is probably the fern’s spore dispersal system. The parallels between the fern’s catapult (a pod called the sporangium) and siege engines of yore go beyond the superficial. There are three physical requirements of a catapult – be it a medieval onager (a siege engine catapult) or a sporangium – for it to be effective. First, there must be a way of storing energy in the catapult, loading the ‘spring’. Second, there must be a trigger: something to release all of the stored energy simultaneously. And finally, there must be a brake: something to stop the ‘arm’ of the catapult at a specific point to send the projectile on its desired trajectory and prevent the arm from crashing into the catapult frame.

Humans can design each of these three features in a siege engine catapult. But how does a fern build such a good catapult without the advantages of engineering? Coraline Llorens at the Nice Sophia Antipolis University, France, and a group of international colleagues used the comparison with a siege engine to explain the mechanisms

meeting each of the three requirements of the fern’s sporangium.

In an onager, the arm is loaded using torsion: the operators twist rope, storing energy in the twists. If the rope were too difficult for the operators to load, the catapult would not work: there must be correspondence between the force applied in loading and the stiffness of the material being loaded. In a fern, the sporangium is loaded by deformation of a row of cells called the ‘annulus’ along the midline of the capsule. Evaporative water loss causes the annulus to bend backwards, storing energy in the bend. Llorens and her colleagues found that the annulus is precisely tuned to deform in response to negative pressure generated by evaporative changes in volume.

The trigger in a fern comes in the form of virtually simultaneous implosion of the annulus cells. This occurs when evaporation causes the negative pressure inside the cells to pass a threshold. This ‘cavitation’ partially closes the sporangium and casts out spores in mere microseconds. But cavitation must be coordinated; if the cells implode too early (before the annulus is loaded) or independently, the catapult would fail. Llorens and colleagues demonstrated that as each cell in the annulus nears threshold pressure, it causes the pressure in the neighboring cells to drop; thus, multiple cells reach the threshold pressure simultaneously.

With an energy storage mechanism and a trigger, a sporangium can launch spores. But it also needs a brake if it wants to avoid spores crashing back into the sporangium itself – and the fern does. The motion of water within the porous annulus cell walls dissipates any remaining stored energy. The movement of water takes considerably more time than the cavitation trigger process, so the sporangium only slows to rest after the spores have been successfully hurled at an appropriate trajectory.

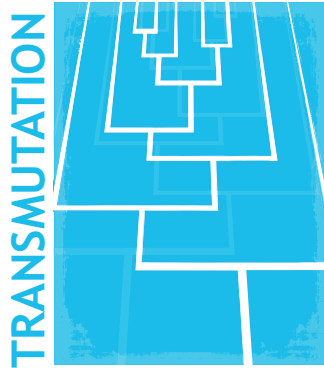
Even though medieval catapults and the sporangium are built in completely different ways, they still share the same underlying mechanical principles. By considering the fundamental requirements of the sporangium, Llorens and colleagues have shown the neat integration of multiple biomechanical systems, to hurl spores with unparalleled speed.

10.1242/jeb.130088

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## The odds of rolling snake eyes



Most vertebrate retinas are composed of two types of photoreceptors: rods, for dim light vision, and cones, for color vision under brighter conditions. However, several interesting exceptions to this duplex organization are seen in the squamate reptiles, a scaly group that contains lizards and snakes. In 1934, Gordon Walls, a post-doc studying visual physiology at the University of Michigan, put forward an inventive theory of visual evolution that sought to explain these exceptions.

Walls observed that some lizards and snakes lacked rods entirely – they possessed a pure-cone retina. These reptiles lived a bright, diurnal lifestyle, and may have had less need for low-light rod vision. However, many evolutionary relatives of the pure-cone reptiles possessed the exact opposite – pure-rod retinas. These reptiles had adapted to a

dim, nocturnal lifestyle. Finally, some squamates had intermediate retinas with eclectic mixes of rods, cones and, occasionally, photoreceptors that resembled both rods and cones.

Based on this diversity, and the evolutionary relationships of pure-rod and pure-cone reptiles, Walls developed a ‘transmutation’ theory of photoreceptors. This theory proposed that rods and cones were not fully independent, but could swap classes through evolution. Unfortunately, Walls had limited means to test his theory, because he lacked a reliable technique to identify the visual pigments, which serve as molecular signatures of the different photoreceptor types.

Two recent papers have revisited Walls’ transmutation theory and found evidence that he was basically on the right track. In the first, Simões and colleagues, writing in the Proceedings of the Royal Society B, used microspectrophotometry and genetic sequencing to identify the visual pigments expressed by a diverse group of snakes – some with all-cone retinas, some with all-rod retinas and others with intermediate photoreceptor types. They found that all-cone retinas still expressed visual pigment genes characteristic of rods. The opposite was also true: all-rod retinas expressed cone visual pigments.

The second study, by Schott and colleagues, writing in PNAS, investigated the garter snake, which possesses an all-cone retina. They looked closely at the ultrastructure and gene expression of these cones, and found that they exhibit many rod-like features, and are thus likely to have evolved from rods. Together, these two studies support Walls’ original theory that rods can transmutate into cones, and cones can transmutate into rods.

But things may be even more complicated than Walls originally predicted. By

combining gene expression studies with phylogenetics, Simões and colleagues suggest that both cone-like rods and rod-like cones have evolved independently at least twice. Indeed, this work, combined with previous studies of the gecko eye, indicates that some reptilian retinas have undergone repeated, radical shifts in photoreceptor composition. This is in contrast to the retinas of fishes, birds and mammals, where the rod/cone dichotomy is thought to have remained relatively static for millions of years.

Why are photoreceptors so evolutionarily fluid in reptiles compared with other vertebrates? One possibility is that squamate visual pigments have somehow been decoupled from photoreceptor morphology, allowing rod pigments to be expressed in cones, and vice versa. Future studies could use transcriptional profiling of intermediate photoreceptors to investigate the genetic mechanisms of transmutation. It remains to be seen whether there is just one or multiple different ways for rods and cones to swap identity. Although many people shiver at the thought, a deep gaze into the eyes of serpents could help us understand how our own retinas ended up with their characteristic duplex structure.

10.1242/jeb.130096

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