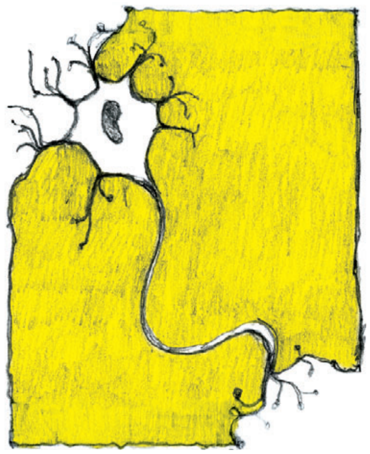
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

SENSORY PERCEPTION



MUTANT MOSQUITOES REVEAL DEET'S DUAL ACTION

Nothing fills a camper's heart with dread like the incessant whine of an active local mosquito population. Mosquitoes are able to track large mammals like humans by sniffing out exhaled carbon dioxide and homing in on the scent of chemicals produced in mammalian skin. While many mosquito species are generalists, happily sucking the blood of any unlucky host, *Anopholes gambiae* specialises on humans. As a result, *A. gambiae* is an extremely efficient vector for malaria, infecting hundreds of millions of people a year with the disease. But a recent study by a team of scientists based in both California and New York has found that mutations in a single gene coding for the olfactory co-receptor Orco can keep *A. gambiae* from both preferring humans and being deterred by the common mosquito repellent DEET.

Postdoctoral researcher Matthew DeGennaro (from Rockefeller University) and his colleagues first created a line of *A. gambiae* mosquitoes that produced non-functional Orco protein by mutating wild-type mosquitoes with zinc-finger nucleases targeted to the *orco* gene. The researchers then ran the mutant mosquitoes through several tests designed to find out which aspects of smelling were affected by losing the ability to produce working Orco.

The researchers first showed that the *orco* mutant mosquitoes were still able to find mammalian hosts, which they prefer over non-mammalian hosts, but only when carbon dioxide was present. This suggests that Orco isn't solely responsible for detecting the preferred mammalian hosts in general. However, the *orco* mutants showed no preference for the smell of humans over that of guinea pigs, even though *A. gambiae* wild-type mosquitoes strongly prefer humans. The researchers concluded that Orco must be essential for *A. gambiae* for distinguishing among hosts.

The mosquito repellent DEET is believed to work by interfering with odorant receptors, and so the researchers hypothesised that *orco* mutants would react differently from wild-type mosquitoes to DEET. They found that while *orco* mutants were similarly attracted to control and DEET-covered human arms, they were repelled from blood feeding on the arms by DEET. This meant that DEET must work to repel mosquitoes by two mechanisms: one based on the olfactory system (of which Orco is a key component), preventing mosquitoes from coming nearby, and one based on another system that prevents the close contact of blood feeding.

It might seem like producing a mosquito that isn't repelled from buzzing around you by DEET is a bit counter-productive to peaceful summer days. But this study reveals not only the mechanisms of how DEET works but also the complexity of insect odour reception, opening up potential targets for developing new insect repellents. Perhaps relief isn't far away after all.

10.1242/jeb.084434

DeGennaro, M., McBride, C. S., Seeholzer, L., Nakagawa, T., Dennis, E. J., Goldman, C., Jasinskiene, N., James, A. A. and Vosshall, L. B. (2013). *orco* mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. *Nature* **498**, 487-491.

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GENETIC MODIFICATION

A PERFECT STORM OF GENES

Since their first production, genetically modified organisms have been a polarizing issue, especially when it comes to using genetically modified organisms for food. Some people consider genetically modified organisms a sensible solution to food production shortages, while others condemn them as anything from inherently immoral to an environmental disaster. Genetically modified Atlantic salmon, carrying a gene that enhances growth, may be the first animal to be approved as a human food source, and as such, heated debate surrounds this fish. Amidst the raging debate, a group of scientists decided to study the potential effects of genetically modified salmon on wild fish populations, should fish escape from farms.

In the wild, Atlantic salmon will sometimes reproduce with the more common brown trout, creating hybrid offspring. In this study, Krista Oke, Peter Westley, Derek Moreau and Ian Fleming from Memorial University in St John's, Newfoundland, Canada, decided to investigate whether genetically modified Atlantic salmon would also reproduce with brown trout, and whether the modified genes would appear in hybrid offspring. Taking this a step further, the researchers wanted to know whether salmon–trout hybrids carrying the modified gene would have the accelerated growth rates typical of their genetically modified Atlantic salmon parent. Finally, how would the hybrids carrying the modified gene interact with wild-type Atlantic salmon, and hybrids without the modified gene?

Using genetically modified Atlantic salmon as both mothers and fathers in crosses with brown trout, the authors established that the salmon and trout would reproduce, and that the modified gene appeared in about half of the hybrid offspring. They also found evidence that there is an advantage to carrying this modified gene. When the brown trout was the mother of the hybrid, the offspring carrying the modified gene had higher survival rates than their siblings without the modified gene. Regardless of whether the genetically modified Atlantic salmon was the mother or father, hybrids carrying the modified gene also grew more quickly than hybrids without the modified gene, or wild-type fish of either species when raised in a typical fish farm setting.

However, things got really interesting when the researchers raised these fish in artificial streams more similar to their natural environment. The fish in these streams had to compete for food and jockey for space just as they would in the wild. Within these streams, the patterns of growth shifted dramatically. Unlike in the fish farm setting, the wild-type fish consistently grew at faster rates than the fish carrying the modified gene. Even more exciting was the discovery that in mixed-species streams, hybrids carrying the modified gene suppressed the growth of both genetically modified and wild-type Atlantic salmon. This growth suppression appeared to be a response specifically to hybrids carrying the modified gene, rather than to hybrids without the modified gene. How hybrids carrying a modified gene suppressed the growth of Atlantic salmon remains unclear, but it may be that these hybrids are more aggressive.

While this research offers no definitive answers in the debate on genetically modified organisms, it is clear that an escaped genetically modified Atlantic salmon could reproduce with a wild brown trout, and produce viable hybrid offspring carrying the modified gene. Even more importantly, this research revealed unexpected consequences of introducing genetically modified fish on natural populations, as hybrids carrying the modified gene suppressed the growth of wild Atlantic salmon. In summary, the findings emphasize that good scientific research is indispensable when assessing the viability of using genetically modified animals.

10.1242/jeb.084442

Oke, K. B., Westley, P. A. H., Moreau, D. T. R. and Fleming, I. A. (2013). Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions. *Proc. R. Soc. Lond. B* doi:10.1098/rspb.2013.1047

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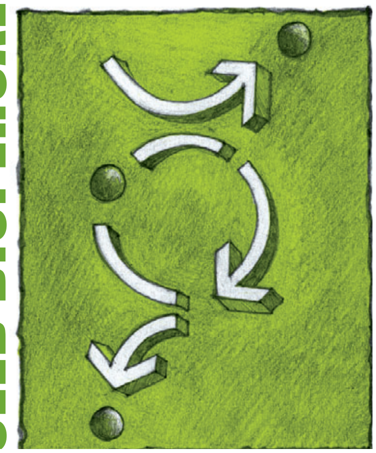
SEED DISPERSAL



BIRDS' DIGESTION CLEANSES PASSING SEEDS

Seeds are born into a hostile and unfriendly world. Animals are eager to devour them while pathogens are keen to infect them. Adding insult to injury, some birds swallow seeds whole and then poop them out far from home. But for some plants, this is exactly the point. In an elegant new study published in *Ecology Letters* by Evan Fricke and an international team of colleagues, the authors show how getting eaten can be a seed's best route to salvation.

Attracted to the fleshy fruits that encase seeds, birds consume fruits and then disperse the gut-processed seeds to new environments. In the early 1970s, ecologists Daniel Janzen and Joseph Connell independently proposed that this benefited seeds, not only because it helped them colonize new habitats but also because it allowed them to escape the species-specific pathogens and predators that were found near their parents. Birds, according to this idea, transport seeds to locations that are free from harm. However, in consuming seeds, birds do far more than disperse them. Seeds are modified during gut passage and it is perhaps these chemical and physical changes that increase seed survival, and not the simple fact that birds deposit the seeds away from the dangers lurking near their parents.

To distinguish these possible benefits, Fricke and his collaborators fed chilli peppers to captive birds and then compared seed survival with that of unpassaged seeds. They found that passage through a bird's digestive system increased seed survival by an astonishing 370%! More surprisingly, this benefit had nothing to do with distance from the parental tree. So what is it about the bird gut that increased seed survival? It turns out that escape from predators and pathogens is crucial after all. Just not in quite the way Janzen or Connell envisaged.

Chilli seeds emit a unique cocktail of volatile odors that are particularly attractive to seed-eating ants. However, after traversing the alimentary canal of birds, these volatiles were reduced ~100-fold, rendering the seeds less detectable by ants. In addition, gut-processed seeds were significantly less infected with fungal pathogens. This roughly doubled seed survival in nature. Granivorous ants are a major seed predator of chilli seeds and *Fusarium* fungal infections are a major cause of seed mortality. Accordingly, the changes to seeds resulting from gut passage have a stunning overall effect on seed survival and plant fitness.

Bird-consumed seeds travel two important paths. Their first short trip through the bird's gut may be inhospitable but offers some predictability. By contrast, their second, longer trip from parental tree to the site of defecation or regurgitation is filled with biotic unknowns. The importance of this study is that it provides a neat link between these two seed voyages: the changes wrought during their first voyage improve survival at their final port of call. It also highlights the novel ways in which birds can mediate the interactions between pathogens, predators and fruiting plants.

10.1242/jeb.084426

Fricke, E. C., Simon, M. J., Reagan, K. M., Levey, D. J., Riffell, J. A., Carlo, T. A. and Tewksbury, J. J. (2013). When condition trumps location: seed consumption by fruit-eating birds removes pathogens and predator attractants. *Ecol. Lett.* **16**, 1031-1036.

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WASP VENOM REGULATES CALCIUM IN FLY HEMATOCYTES

Parasites have evolved to attack host immune systems using a variety of weapons known as virulence factors. In addition to helping parasites evade host defenses, virulence factors can also help us understand how immune systems function. In a recent edition of *PNAS*, Nathan Mortimer and colleagues from Emory University uncovered previously unknown immune mechanisms by exploring how certain parasitoid wasp virulence factors overwhelm fruit fly defenses.

Parasitoid wasps inject eggs and venom into invertebrate hosts; the eggs hatch and the larvae then consume the host before emerging as adult wasps. But not all hosts are passive victims; larval fruit flies, for example, mount an effective response. After infection by a wasp, the fly immune system up-regulates blood cell production and encapsulates the egg with specialized blood cells. To better understand the mechanisms of this response, Mortimer and colleagues enlisted the help of a little-studied parasitoid fly, *Ganaspis sp.1*. These wasps are interesting because they are so deadly. The team found that adult *Ganaspis* emerged from nearly 100% of infected animals in over a dozen species of fruit flies. This is in contrast to other 'avirulent' wasp species (e.g. *Leptopilina clavipes*) that have emergence rates of only a few per cent after infection.

To address whether *Ganaspis* evades or actively suppresses the fly immune system, the team used mutant flies that spontaneously encapsulate their own tissues. When these mutants were infected by *Ganaspis*, self-encapsulation was reduced. Furthermore, after *Ganaspis* attack, wild-type fly larvae showed the same increase in blood cell production that accompanies infection by *L. clavipes*. These results show that fly larvae try to mount a response to infection by *Ganaspis*, but a virulence factor prevents them from encapsulating the eggs.

To understand how *Ganaspis* disables encapsulation, Mortimer and colleagues first used transcriptomics and mass spectrometry to identify the most abundant proteins in wasp venom. Surprisingly, the most common venom protein is a large pump (sarco/endoplasmic reticulum ATPase, SERCA) that downregulates intracellular calcium by moving it into internal stores. This prompted the team to examine calcium levels in isolated blood cells in response to wasp venom. Wasp venom triggered a decrease in calcium in one type of blood cell (plasmatocyte). This response was abolished by addition of a pharmacological inhibitor of SERCA. The team also compared calcium levels in plasmatocytes after attack by *L. clavipes* and *Ganaspis*. Attacks by *L. clavipes* triggered a transient burst of calcium in plasmatocytes; these calcium spikes were not present in plasmatocytes after *Ganaspis* attack. When these calcium bursts were genetically inhibited, larvae lost the ability to encapsulate the normally easy-to-handle *L. clavipes* eggs. In contrast, genetically increasing intracellular calcium levels enhanced the number of larvae that encapsulated the tough-to-tackle *Ganaspis* eggs. Overall, these experiments show that bursts of calcium in plasmatocytes are required to initiate the immune response to wasp attack. They also show that the reason why *Ganaspis* is so deadly is because it has a calcium pump in its venom that prevents the calcium-mediated response.

The work of Mortimer and colleagues demonstrates the value of taking a comparative approach to the study of immune system function. By studying a diverse array of co-evolving parasites and their hosts, researchers can uncover new mechanisms of immune function.

10.1242/jeb.084459

Mortimer, N. T., Goeks, J., Kacsoh, B. Z., Mobley, J. A., Bowersock, G. J., Taylor, J. and Schlenke T. A. (2013). Parasitoid wasp venom SERCA regulates *Drosophila* calcium levels and inhibits cellular immunity. *Proc. Natl. Acad. Sci. USA* **110**, 9427-9432.

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