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New antibody insecticide targets malaria mosquito

African malaria mosquito, *Anopheles gambiae*. Photo credit: Benjamin Krajacich.

While the carnage wreaked by the current Ebola epidemic in West Africa has played out in the gaze of the world's media, another pandemic has continued virtually unnoticed in the background. Malaria threatens half of the human population and even now kills one child every minute. Yet mortality rates from malaria have been falling steadily since 2000, thanks to insecticide-treated bed nets and spraying programmes targeting the African malaria mosquito (*Anopheles gambiae*), which spreads the disease. However, the mosquitoes are fighting back. Resistance to the pesticide pyrethroid is increasing, leading scientists to search for other effective insecticides. 'Ivermectin has arisen as a new candidate,' says Jacob Meyers from Colorado State University, USA, explaining that the drug kills or disables *A. gambiae* mosquitoes after consumption in a blood meal. However, little was known about how Ivermectin targets mosquitoes – it was developed to treat diseases in nematodes – so Meyers, Brian Foy and a team of colleagues from Colorado State University, including Meg Gray, Wojtek Kuklinski, Lucas Johnson, Christopher Snow, William Black IV and Kathryn Partin, decided to find out more about the drug's *modus operandi*.

Explaining that Invermectin targets and opens a key component of the synaptic communication system – the glutamate gated chloride channel (GluCl) – to kill nematodes, the team decided to find out more about the channel's function in the mosquitoes at a genetic and physiological level. Cloning the mosquito gene for the GluCl channel (AgGluCl) and analysing its structure, Gray, Black and Meyers were surprised to find that it could be expressed in four different ways, producing subtly different versions of the chloride channel – known as splice isoforms. However, after months of attempts to measure the minute currents flowing through the four channel isoforms in the presence and absence of Ivermectin, Partin and Meyers were in for a shock. 'We discovered one channel [AgGluCl-a] was sensitive to Ivermectin, as predicted, but the second isoform tested [AgGluCl-b] was surprisingly insensitive', says Meyers. He adds that the lack of sensitivity is puzzling, because Ivermectin should bind successfully to both channels to activate them – the region of AgGluCl-a that interacts with the drug should be identical to the Ivermectin binding site in AgGluCl-b.

Next, Kuklinski and Meyers analysed the expression patterns of the different channel isoforms and were pleased to see that the 'a' forms of the protein were expressed predominantly, explaining the insect's vulnerability to the drug. However, the team warns that the cunning mosquitoes could develop immunity to the insecticide if they switched expression to produce the Ivermectin-insensitive 'b' form of the channel.

Finally, Meyers tested which tissues the channel was expressed in and located it in nerves, including the thoracic ganglia, controlling the mosquitoes' movements. Recalling that Ivermectin causes paralysis, he says, 'Our data suggest that this paralysis may be due to disruption of AgGluCl in the motor neurons controlling the leg and flight muscles'.

Having narrowed down how Ivermectin targets the AgGluCl channel to incapacitate malaria-spreading mosquitoes, Foy and Meyers wondered if they could find an even more effective way of defeating the African malaria mosquitoes by targeting the essential channel using another strategy. Could they make blood meals toxic for the voracious mosquitoes by immunising the animals that they feast upon to produce antibodies targeting the AgGluCl channel? And if so, could such a therapy be used to target other disease-carriers, such as yellow fever mosquitoes and West Nile virus mosquitoes? Meyer admits that the strategy was risky. 'Antibodies against a single mosquito antigen have never been shown to have mosquitocidal properties before and the majority of previous research had focused on midgut antigens, while we were targeting a neuronal antigen expressed only in tissues found outside of the midgut', he says.

Injecting rabbits with a tiny portion of the surface of the protein channel, Meyers waited for the animals' immune systems to kick in and begin producing antibodies tailored to the channel. Then he collected the antibodies, mixed them with fresh blood and fed the tasty mixture to all three mosquito species.

Frustratingly, neither the yellow fever nor West Nile virus mosquitoes reacted to the spiked blood. However, significant numbers of the malaria mosquitoes expired after the blood–antibody cocktail, with the highest antibody doses killing over 90% of the insects within a day. And when Meyers and Gray tested why the yellow fever and western encephalitis mosquitoes had been immune to the antibody snack, they found that the antibodies could not pass across the guts into the haemolymph of the yellow fever or West Nile virus mosquitoes, while the antibodies passed into the haemolymph of the malaria-carrying mosquitoes with ease.

Intrigued by the antibodies' attack mechanism, Meyers fed the insects a blood meal laced with the antibodies and a lethal dose of Ivermectin and monitored their survival. Remarkably, the insects survived much better than insects fed Ivermectin alone. 'We believe that Ivermectin is still able to bind to AgGluCl, but the antibody keeps the

channel from opening, even after Ivermectin binds to it', he says.

Having shown that antibodies targeted to AgGluCl in blood meals can be effective insecticides, Meyers and Foy are keen to find out if antibody-laced blood meals are equally deadly in real life. 'The next step… is to immunize cattle against the AgGluCl antigen and directly feed *A. gambiae* on the immunized cattle in the lab', explains Meyers. And if the strategy proves successful, Meyers envisages a large-scale cattle immunisation program as part of a combined attack on the parasite. 'Cattle are a major blood meal source for multiple malaria vectors,' he says, explaining that any malaria-harbouring mosquito that consumed blood carrying the toxic antibodies during the malaria parasite's incubation period would die, disrupting transmission of the disease and offering hope of a malaria-free future for generations to come.

10.1242/jeb.124412

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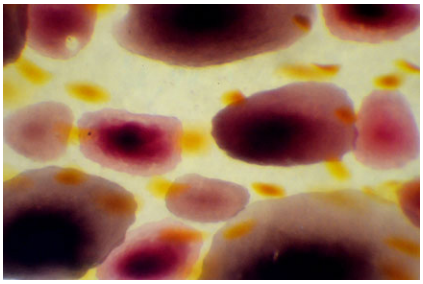
Kathryn Knight

Cephalopods sense light with skin



Chromatophores in the skin of *Doryteuthis pealeii*. Photo credit: Alexandra Kingston.

Masters of disguise, many cephalopods think nothing of changing their skin colour to blend in with the surroundings. 'These changes primarily rely on eyesight', say Desmond Ramirez and Todd Oakley from the University of California, Santa Barbara, USA, who explain that octopuses collect information about their setting with their large camera-like eyes before sending signals to chromatophores in the skin to change colour. However, Ramirez had noticed two reports describing how the colour-changing structures (chromatophores) in tiny biopsies of squid and octopus skin reacted to light with no input from the eyes or brain; although no one had followed up on the observations. Meanwhile, Alexandra Kingston and Thomas Cronin from the University of Maryland, Baltimore County, USA, were also pondering the possibility that cephalopod skin may respond to light because other biological structures are known to detect light. In addition, they also knew that proteins – so-called non-visual opsins that are analogous to the opsin proteins that sense light in eyes – are produced in the skin of various animals. Intrigued by the possibility that cephalopod skin is sensitive to light, both teams embarked on independent studies to see if they could identify key components of the light-sensing mechanism found in eyes in the skins of octopus, squid and cuttlefish.

Collecting skin biopsies from California two-spot octopuses, Ramirez and Oakley first shone white light on the tissue and were impressed to see the colour-changing chromatophores expand when light fell on them and relax when the light went off, returning the skin to its original hue. Then, they measured how long it took the chromatophores to expand to change the skin's colour when exposed to light ranging in colour from violet to orange, finding the swiftest response to 480 nm light (blue), which coincides with the wavelength of light that the octopus's eye opsin responds to most strongly. Referring to the light reponse as light-activated chromatophore expansion (LACE), the duo then tested the skin for evidence of expression of opsin genes, and they were pleased to find expression of the gene for rhodopsin, the opsin protein that is usually produced in the eye. And when the duo tested where the rhodopsin protein was produced in the skin, they found it localised to sensory neurons distributed on the mantle surface.

Focusing on several cephalopods – two cuttlefish and a squid – Kingston and Cronin decided to investigate whether they could identify components of the molecular machinery that is essential for converting light detection into a behaviour. As they knew that light activates rhodopsin in the retina, triggering a cascade of protein interactions that culminate in an ion channel opening to signal light detection, the pair of scientists began searching for key proteins in the rhodopsin signalling cascade in the skin of the common cuttlefish, the broadclub cuttlefish and the longfin inshore squid. Using a battery of molecular techniques, Kingston and Cronin identified rhodopsin in the skins of both cuttlefish and the squid, and they also found other proteins that are essential for the light sensing in the animals' skin. Next, they teamed up with Alan Kuzirian and Roger Hanlon to look in closer detail at chromatophores isolated from different regions of the squid's mantle and found that the light-sensing cascade proteins were actually in the chromatophores. 'The very same structures that show behavioral responses are actually themselves light-sensitive', says Cronin.

So, both teams present compelling evidence that cephalopods may have adapted the cellular mechanism that detects light in eyes for light sensing in skin, and Ramirez and Oakley have collected the first recording of the skin's sensitivity across the visual spectrum. In addition, Kingston and Cronin suggest that squid chromatophores directly sense the light that they respond to through three possible mechanistic scenarios: they could interact directly with adjacent cells, through the gap junctions between muscle cells or through nerve fibres to communicate with the central nervous system, providing information about the animals' surroundings while it tries to blend in.

10.1242/jeb.124438

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Kathryn Knight

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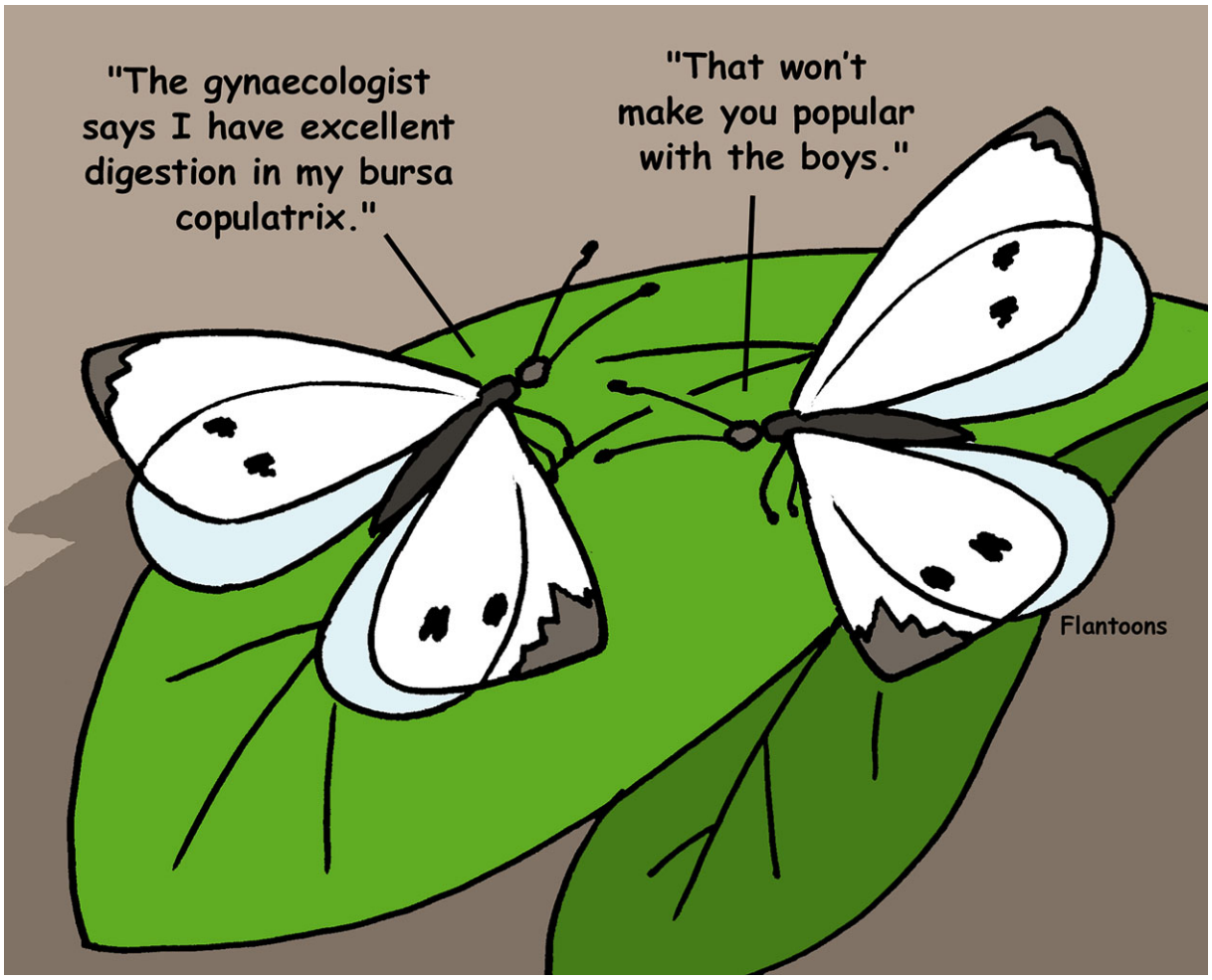
Female butterflies digest sperm packages in love duel



When the Elizabethan dramatist John Lyly coined the phrase, 'All is fair in love and war', butterflies were probably the last thing on his mind. But, in the never-ending battle between the sexes, the sentiment is tailor-made for these duelling mates. Melissa Plakke and colleagues from the University of Pittsburgh, USA, explain that ejaculating male butterflies deliver their sperm in a package, called the spermatophore, containing other compounds that probably nourish the female, although this may sometimes be at her expense. However, in the spirit of combat, the females have fought back, developing a specialised organ – the bursa copulatrix – designed to release the sperm rapidly and then dispose of the remaining package with grinding structures and protein-digesting enzymes in a bid to mate with as many males as possible. Plakke says, 'Little was known about how the bursa digests the spermatophore', so the team began investigating the bursa of small cabbage white butterfly virgin females in various social situations and recently mated females to find out how they break down spermatophores.

Painstakingly extracting proteins from the butterflies' bursal tissue, the team found the reproductive organ produced high levels of proteolytic enzymes: by 3 days after emergence, the protein-digesting capacity of the females' tiny bursas surpassed the protease activity of the large intestines of hungry caterpillars. However, after mating, the protease levels in the bursas of recently mated females initially fell, increasing again 5 days later. And when the team investigated which protein-degrading enzymes the tissue was producing, they identified nine proteases.

Admitting that they are impressed that the bursa copulatrix (~1 mg) is capable of producing as much protease activity as the 20 mg caterpillar midgut, the team also points out that the enzyme activity varies wildly over time, suggesting that both the males and females may contribute to protease regulation. They also suggest that instead of producing proteases on demand, the females accumulate and store large amounts of the enzymes in the bursa, placing them at risk of internal damage, although they suspect that the females mate early and frequently enough to protect them from self-harm.

10.1242/jeb.124420

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Kathryn Knight
kathryn@biologists.com