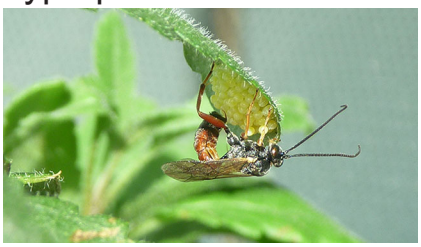
INSIDE JEB

Wolbachia makes parasite vulnerable to hyperparasite



Hyposoter horticola wasp laying eggs in *Melitaea cinxia* eggs. Photo credit: Anne Duplouy.

Evil parasites are the stuff of horror movies, bursting out of unsuspecting victims in the most ghoulish fashion possible. However, it seems that even parasites are not immune to parasites of their own. Anne Duplouy, Saskya van Nouhuys and Minna Kohonen from the University of Helsinki, Finland, describe how *Hyposoter horticola* parasitic wasps, which exclusively target Granville fritillary butterfly caterpillars – on the Åland Islands off Finland – are susceptible in turn to *Mesochorus* cf. *stigmaticus* hyperparasitic wasps, which hijack *H. horticola* larvae as incubators for their own offspring. However, Duplouy had also noticed previously that *H. horticola* populations had lower levels of *M.* cf. *stigmaticus* infection where *H. horticola* had high rates of infection by the symbiotic *Wolbachia* bacteria (doi: 10.1371/journal.pone.0134843). Explaining that *Wolbachia* infections are often beneficial to their hosts in order to maintain their welcome, Duplouy wondered whether the bacteria also boosted the resistance of *H. horticola* to *M. stigmaticus* hyperparasitic lodgers – consequently reducing *M.* cf. *stigmaticus* infection rates when *Wolbachia* infection rates are high. However, there was an alternative explanation for low levels of *M.* cf. *stigmaticus* infection: if *Wolbachia* reduced the resistance of *H. horticola* to *M.* cf. *stigmaticus*, this would allow *M. stigmaticus* to kill off *Wolbachia*-infected *H. horticola* to keep their numbers down when *M.* cf. *stigmaticus* infection rates were high.

Intrigued by both possibilities, the team investigated whether *Wolbachia*-infected *H. horticola* were better at evading *M.* cf. *stigmaticus* infections or became more susceptible to the tenacious hyperparasite. First, they had to produce *H. horticola*-infected Granville fritillary caterpillars that they could place in the environment, where half of the caterpillars were carrying *H. horticola* that were infected with *Wolbachia* and the other half were *Wolbachia* free.

Duplouy and colleagues collected parasitized fritillary larvae from the Åland Islands and waited for the adult *H. horticola* wasps to emerge. Knowing that only 50% of the parasitic wasps carried *Wolbachia*, they then offered clutches of butterfly eggs to the *H. horticola* wasp mothers for them to lay their eggs in. After nurturing the butterfly eggs as they developed into caterpillars, the team checked that some individuals from each clutch were carrying *H. horticola* wasp larvae and then transferred the parasitized caterpillars onto potted plants, where they spun silky nests for overwintering; the team finally transferred the plants and their parasitised caterpillars to Åland Islands to measure the impact that *Wolbachia* infection had on the rate of *M.* cf. *stigmaticus* infestation of *H. horticola* larvae.

Retrieving the caterpillar nests from the islands several weeks later and comparing the number of butterfly nests that had been targeted by the *M.* cf. *stigmaticus* hyperparasites, the team found that 74% of the *H. horticola* larvae that carried *Wolbachia* were infested by *M.* cf. *stigmaticus* larvae, in contrast to the *Wolbachia*-free *H. horticola*, which only suffered a 40% *M. stigmaticus* infestation rate. So, *Wolbachia* infections seem to make *H. horticola* larvae more vulnerable to parasitic infection, which poses the question of why *H. horticola* maintains the close relationship with symbiotic bacteria that increase its vulnerability in some circumstances. 'In order to persist in the host population, [*Wolbachia*] must have a positive effect on the fitness of infected parasitic wasps that could outweigh the costly burden of susceptibility to widespread parasitism', says Duplouy and colleagues, who suspect that bacteria may contribute to *H. horticola*'s resistance to other parasitic infections instead.

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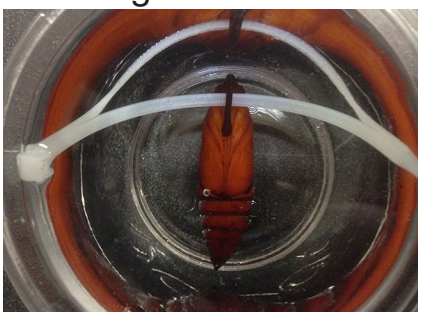
van Nouhuys, S., Kohonen, M. and Duplouy, A. (2016). *Wolbachia* increases the susceptibility of a parasitoid wasp to hyperparasitism. *J. Exp. Biol.* **219**, 2984–2990.

Kathryn Knight

Manduca larvae survive drowning



Submerged *Manduca sexta* pupa. Photo credit: Steve Lane.

Dragonfly larvae are perfectly equipped for a life of total immersion in their pond homes prior to emergence for their final metamorphosis. However, other species may be less well prepared for soggy starts in life. Art Woods from the University of Montana, USA, explains that although the larvae and pupae of other insects spend some portion of their lives buried in dry soil, their conditions can change rapidly when the weather turns. Whether it's the onset of the monsoon or a rapid thaw, dry soil in river beds and desiccated flood plains can flood quickly, submerging larvae as the soil saturates. 'Anoxia can be highly stressful, even in highly anoxia-tolerant individuals', says Woods, who was intrigued by how well *Manduca sexta* moth pupae survive drowning.

Graduate student Steven Lane submerged pupae for up to a fortnight and plucked the insects from the water on alternate days (from day 1 to 13) to find out whether they survived and, if so, how long it took

them to recover from total immersion. Impressively, all of the pupae that were removed from the water after submersion for 5 days survived; however, the partially drowned pupae took 7 days longer to emerge as adults than pupae that had remained dry, and extending the immersion by 2 days proved fatal. 'Survival times of pupal *M. sexta* in immersion (anoxia) are impressive but not unprecedented', says Woods, adding that other species that suffer flooding are capable of surviving similar periods. And when Lane measured the amount of lactic acid produced by the submerged insects, the pupae that had been submerged for longest had the largest amount and it took 2 days to dissipate; the insects had switched from aerobic to anaerobic respiration while submerged.

Recording the pupae's respiration patterns as they recovered, it was clear to Lane that they initially opened their spiracles for an extended period to release large amounts of CO_2. However, once the CO_2 levels had fallen sufficiently, individual spiracles began to close, possibly because the pH of the body tissues had increased as the CO_2 seeped out. And then the CO_2 emission pattern switched again, rising and falling every 0.8–2.2 min, suggesting either that the pupa was opening and closing its spiracles rapidly or that the insect was pumping its abdomen while holding its spiracles open to expel the accumulated CO_2. It was also clear that the recovering insect's metabolic rate was 50–75% higher than that of pupae that had not been submerged. As the spiracle opening patterns that produce intermittent breathing patterns in insects in other circumstances are regulated by the interplay between CO_2 and O_2 levels in the spiracles and adjacent tissues, Woods explains that only one scenario – where spiracle opening and closing are triggered by the acidity of the surrounding tissue – seemed likely to produce the conditions that could trigger some, but not all, aspects of the recovering pupae's unusual high-frequency breathing pattern.

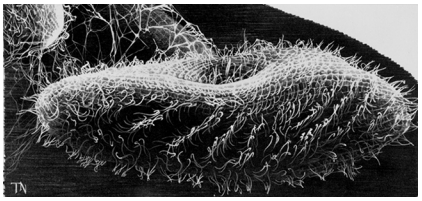
So, the pupae of species that occasionally suffer flooding and are at risk of drowning are capable of recovering after several days of submersion, and Woods says, 'Pupae of *Manduca* would make a good model system for further studies linking immersion, anoxia tolerance and the mechanisms underlying patterns of gas exchange in insects'.

10.1242/jeb.149591

Woods, H. A. and Lane, S. J. (2016). Metabolic recovery from drowning by insect pupae. *J. Exp. Biol.* **219**, 3126–3136.

Kathryn Knight

Ca_V channels send *Paramecium* into reverse



Paramecium with 10 µm-long cilia from Grass Calendar, 1985. Picture credit: Judith Van Houten.

The tissues and organs of most animal bodies are lined by ceaselessly beating microscopic hairs – from the structures that waft cerebrospinal fluid around the brain to lung-cleansing cilia, and many single-cell organisms, such as *Paramecium*, use similar structures for propulsion in the pursuit of food and to avoid difficult conditions. Junji Yano and Judith Van Houten from the University of Vermont, USA, explain that the relentlessly beating motion of *Paramecium* cilia is driven by calcium currents passing through protein channels within the microscopic structures that switch the direction in which the cilia beat to send the cell into reverse. The true identity of the proteins that comprise these channels had remained elusive for almost half a century until Van Houten's team identified components of the calcium channel in the cilia of *Paramecium tetraurelia* in 2013. But there was still no direct evidence that these channels were the origin of the essential calcium current, so Yano, Sukanya Lodh and Van Houten set about directly testing the role of the channel in *Paramecium* swimming behaviour.

RNA interference (RNAi) – where segments of RNA that match the sequence of a target gene are fed to organisms to prevent production of the protein that is encoded by the gene – is a powerful technique that allows scientists to assess the physiological role of specific genes. As Van Houten's team had previously sequenced three calcium channel alpha 1 subunits (Ca_V1a, 1b, 1c), Lodh and Yano produced RNA fragments containing lengthy sections of the three calcium channel subunits, fed them to *P. tetraurelia* for up to 72 h and then transferred the cells to a solution that triggers swimming in reverse to assess how losing channel components affected swimming performance. Comparing *Paramecium* cells that had received the RNAi diet with those that had not, the team found that cells that had lost one channel subunit could only reverse for ∼3–5 s and cells that had lost all three barely reversed at all (∼2 s); however, cells that not been fed RNAi swam backward for ∼23 s. The Ca_V channel proteins found in the cilia were responsible for *Paramecium*'s about-turn.

However, the team needed further convincing that the Ca_V1a–c channel subunits provided the essential calcium current, so they turned their attention to a mutant form of *P. tetraurelia* – known as 'Pawn' – which, just like its chess piece namesake, is unable to reverse. As there were two possible explanations for the Pawn mutants' inability to reverse – either they lacked Ca_V channels in their cilia or they had the channels, but the proteins were inactive for some reason – Lodh and Yano added a tag to the end of the Pawn Ca_V1c gene and allowed the cells to grow before collecting the cilia. Then they tested for evidence of the tagged channel protein in the cilia, but there was none, suggesting Pawn mutants lack Ca_V channels in their cilia.

Lodh and Yano then inserted normal copies of the genes that are known to be defective in Pawn cells (known as *PWA* and *PWB*) and tested the modified cells; not only had they regained the ability to reverse but also the essential Ca_V channel proteins were present in the cilia. In addition, the normal PwB protein appears to be associated with the Ca_V1c protein in the endoplasmic reticulum, where proteins are synthesised, suggesting that PwB may be involved in transporting Ca_V1c to the cilia, where the protein contributes to production of the calcium current that is essential for the change in direction of *P. tetraurelia*'s beating cilia.

10.1242/jeb.149724

Lodh, S., Yano, J., Valentine, M. S. and Van Houten, J. L. (2016). Voltage-gated calcium channels of *Paramecium* cilia. *J. Exp. Biol.* **219**, 3028–3038.

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Journal of Experimental Biology (2016) 219, 2965-2967

Testosterone regulates scarlet plumage in red-backed fairy-wrens

As the days begin to lengthen and winter recedes for yet another year, many birds dust off their most glamorous plumage to attract a mate. Males often opt for ostentatious displays while females tend to the dowdier end of the spectrum. But what factors regulate the dramatic differences in avian regalia between males and females? Willow Lindsay from the University of Gothenburg, Sweden, explains that the hormones that regulate sexual differences were thought to play a key role in defining plumage patterns, but the precise role of individual hormones was less clear. As testosterone starts flowing in spring, male red-backed fairy-wrens (*Malurus melanocephalus*) shed their dreary brown feathers in favour of a vivid scarlet and glossy black coat, while the females retain their dingy brown and white plumage. So, Lindsay and Douglas Barron headed south to Queensland, Australia, to find out how the plumage of red-backed fairy-wrens altered after they received a testosterone implant.

Initially, the males and females both looked drab; however, soon after the birds received the implant, the females moulted and began sprouting shorter male-like feathers. The females also produced a vibrant patch of red feathers on their backs, similar to the bright red streak on the males' backs. In addition, the feathers on the crown, belly and breast of the females turned a shade of pinky-orange, which Lindsay said was: 'Never previously documented on a red-backed fairy-wren'. Only one female went on to produce black feathers like those of the males, although the beaks of all of the females became darker. And when Lindsay and Barron injected some females with gonadotropin-releasing hormone, in a bid to naturally raise the birds' testosterone levels, the females were unable to increase the levels of the hormone in their blood.

So, it seems that testosterone is the hormone that regulates the production of the carotenoid pigments that produce the males' scarlet plumage; however, the hormone was unable to elicit production of the jet-black melanin pigment that produces their distinctive dark feathers. It seems that the female fairy-wrens still maintain the essential hormone mechanism that allows the males to put on their gaudy display, although the females are unable to produce sufficient testosterone to trigger the masculine transformation.

10.1242/jeb.149609

Lindsay, W. R., Barron, D. G., Webster, M. S. and Schwabl, H. (2016). Testosterone activates sexual dimorphism including male-typical carotenoid but not melanin plumage pigmentation in a female bird. *J. Exp. Biol.* **219**, 3091–3099.

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