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INSIDE JEB

Tobacco hornworm caterpillars held up by imaginal disc damage

A tobacco hornworm (*Manduca sexta*) caterpillar. Photo credit: Fuse lab.

Youngsters often bear more than a passing resemblance to their parents; but, the offspring of other creatures look nothing like their forebears. 'Think maggot to fly, or caterpillar to butterfly or moth', says Megumi Fuse, from San Francisco State University, USA; their family resemblances only become apparent when fully formed adults emerge from their final metamorphosis. Yet, the apparent absence of limbs during early insect development does not mean that they are missing entirely. Even the most underdeveloped maggots have structures – known as imaginal discs – that eventually go on to develop into limbs and essential appendages. However, the ability of these proto-limbs to develop rapidly also places maggots at risk if their imaginal disc structures are damaged; the harm can be transmitted to newly developing limbs. One insect, *Drosophila*, overcomes such setbacks by simply shutting down development, giving damaged disc structures time to repair and regenerate. But the tiny maggots are notoriously tricky to investigate, so Fuse and her team of dedicated undergraduate and Master's students wondered whether heftier tobacco hornworm (*Manduca sexta*) caterpillars could help them understand more about the brakes that imaginal disc damage might put on the mighty moth.

First, Fuse wanted to know how the caterpillars responded if the imaginal discs that eventually develop into four wings were damaged. Brenda Cisneros Larios poked the structures with forceps and monitored the caterpillars as they transformed into pupae (the moth equivalent of butterfly chrysalises) before emerging as adults. Sure enough, the damage to the wing imaginal discs delayed the adults' emergence and, when Nicholas Silva exposed the caterpillars to X-rays – which only damage the rapidly developing structures – the injury delayed the insects' development by up to 3 days. Teaming up with Ben Abdon and Tigran Makunts, Silva also confirmed that the radiation had damaged the imaginal discs' DNA. So, the large caterpillars were able to delay their development, but how were the insects coordinating the hold-up?

Knowing that a pair of key hormones, ecdysone and juvenile hormone, are key factors that trigger the transformation into adults for *Drosophila* maggots, Abdon spent several months collecting daily haemolymph (insect blood) samples to find out whether a dose of X-rays affected when the injured caterpillars produced ecdysone. And, when Ernest Chang analysed the minute blood samples, the damage had delayed the surge of ecdysone just like it does in *Drosophila*, slightly postponing the caterpillars' transformation. However, when Manuel Rosero and Jhony Zavaleta checked the mass that the caterpillars must attain before they commit to transforming (their critical mass), the X-rayed caterpillars had to reach 7.5 g, compared with their un-X-rayed siblings, which committed to their metamorphosis at 5.5 g. And when Rosero analysed the injured caterpillars' juvenile hormone levels – which usually fall and trigger metamorphosis when uninjured animals reach their critical mass – the hormone levels dropped sooner than the team had expected, which was surprising given that their metamorphosis was slightly delayed. 'It was always thought that [pupal] commitment depended on when the juvenile hormone levels dropped', explains Fuse.

So, although tobacco hornworm caterpillars are able to delay development to allow their imaginal discs to recover, the delay seems to be via a different mechanism from that which allows injured *Drosophila* maggots to buy time. And Fuse adds, 'I would be very excited to try to address developmental delays in a number of insects to see just how specialised the delays are'.

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