





## The caterpillars who see through their skin to better blend in

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## ABSTRACT

Peppered moth caterpillars are masters of masquerade. They look and act like the twigs in their environment, even changing colour to create a closer likeness. Remarkably, they can still do this when blindfolded. This ability is suggestive of colour-sensitive visual machinery outside of the eye that allows larvae to stay camouflaged in rapidly changing environments.



Image credits: Arjen Van't Hof ©

If you think life is hard, try being an insect. In a world where everything is out to eat or kill you, merely staying alive is a challenging task. Therefore, it's no surprise that insects have evolved several different ways to avoid being eaten by predators. A popular strategy is camouflage:

hiding in plain sight. The <u>story</u> of how the peppered moth camouflaged itself against the trees during a changing environment is a famous one and a textbook example of natural selection. How the caterpillars of this species remain undetected, is more of a mystery.

Peppered moth caterpillars show a remarkable resemblance to the twigs they rest upon, right down to their gnarled appearance and their perfectly still, straight resting posture. But these masquerade masters go one step even further; they change their body colour to match the colour of the sticks in their environment. In 2009, it was <u>proposed</u> that the larvae might be perceiving the colour of their resting background to switch between green and brown morphs. Building upon this study, we later <u>found</u> that larvae were using visual cues to produce a continuous range of colours, including black and white. This evidence got us thinking. A level of colour-matching so diverse and accurate would surely require sophisticated colour vision, but up until now no one really understood– what and how do these caterpillars see?

We began tackling this question by first looking at opsin genes, which code for the light-sensitive proteins required for colour vision in insects. We discovered six types of opsin, each one sensitive to a different colour, or wavelength of light. We also





characterised and measured the expression of two other visual genes. We were surprised to find that these genes were expressed in all skin segments. Some of these genes even showed equal or higher expression in skin tissue compared to the head, where eyes are located. This result yielded another question: Why are these visual genes expressed here - can peppered moth caterpillars sense colour through their skin?

To test this experimentally, we needed to eliminate vision from the eyes of the caterpillars. We decided that the most humane way to do this was to paint over the eyes with acrylic paint. Once dry, it made a tiny black blindfold, not unlike the kind of sleeping mask you'd wear on a long-haul flight. Peppered moth larvae moult 4-5 times until pupation, each time shedding their head capsule. This meant regular repainting, but that the treatment was reversible and the emerging moths could once again see through their eyes. We then placed groups of control (unpainted eyes) and "blindfolded" caterpillars onto brown, green, white, and black painted wooden dowels to see if the blindfolded larvae could still change colour. They could, and surprisingly they colour-matched their dowels as well as the control groups.

Colour change in peppered moth caterpillars can take weeks to complete, and during this time they are vulnerable to predation. This made us wonder if, like some other animals, the caterpillars would prefer to rest on backgrounds that matched their own body colour to decrease their visibility. To test this, we created arenas containing two dowels: brown and green. We placed the same groups of larvae from the blindfolding experiment inside these choice chambers, and after 12 hours observed their final resting position. Brown larvae did indeed rest more frequently on brown dowels than green, and vice versa for green larvae. Blindfolding the larvae did not affect this behaviour.

When combined, the results of these experiments indicate that peppered moth larvae can sense colour through their skin and somehow use this information to match the colour of their environment. This is achieved through either changing their body colour or resting on colour-matching twigs; or a combination of both. Other insects, reptiles and many marine animals change colour, also for camouflage. This appears to be one of the first examples showing camouflage guided by colour vision outside of the eye, but it's very unlikely that peppered moth caterpillars are alone in this ability. We expect that the skin of the larvae houses the light-sensitive cells that mediate colour change, but we would like to determine their exact location. Investigating how widespread this phenomenon is in other moths and butterflies, as well as more distantly related species, will help identify the evolutionary origins of this extraordinary behaviour and the physiological machinery that facilitates it.