

INSIDE JEB

Sleeping butterflies have spirited goo



A direct-developing pupae of the green-veined white butterfly, *Pieris napi*.
Photo credit: Mikael Carlsson.

Butterfly metamorphosis is an iconic transition – an ugly caterpillar shrouds itself in a chrysalis to later emerge as a beautiful butterfly. However, in reality, it's a bit less elegant than some might imagine. 'You have a larva in a pupal casing that turns to goo,' says Philipp Lehmann, at the Stockholm University in Sweden. 'And that is then used to build the adult inside the pupa.' This transition happens directly for some species, but for others, the goo lays dormant – sleeping through winter – until it gets a cue to wake up and make an adult form. Lehmann is fascinated by what's happening in this goo throughout the phases of dormancy, called diapause. This goo, 'an energy-rich meat broth,' says Lehmann, contains lipids and the metabolome – a collection of small-molecule chemicals, including sugars and amino acids – that Lehmann says are the result of many, many metabolic pathways. Lehmann and other researchers had previously looked for changes in the lipids during diapause, and found that not much happens. But, 'We had trouble believing this would be the case with the metabolome,' Lehmann says. So he and an international team

set out to see what the chemicals in the goo were doing in the sleepy pupa.

Lehmann and his colleagues raised green-veined white butterflies in the lab and separated them into three groups. One group they allowed to develop directly from larva to butterfly, and in the other two they triggered diapause by subjecting the larvae to shortened day lengths. They initially held all three groups at a warm temperature, then moved one diapause group to cold, then colder temperatures, for a period of 5 months to cue them to end diapause. This period of cold is necessary in the population of green-veined whites that Lehmann studies. He says, 'Just like seed dormancy in plants, there's a temperature that you must dip under before you kickstart the process we call diapause termination.' Diapause termination is a not a point in time, but a process that requires months at low temperatures. The animals in diapause that were kept warm never began that process, they simply remained in diapause.

The team sampled the animals' metabolome several times throughout

their development. They found that although the sugar and amino acid collection changed in all animals, it changed most in the diapause groups, and most dramatically in the diapause termination group. 'It was so very surprising,' Lehmann says. 'The majority of metabolites were changing very, very much during this otherwise static phase.'

'If you would look at these pupae over this whole experiment, they're just in the fridge in a cup, in complete darkness,' Lehmann explains. 'Nothing happens. Metabolic rate doesn't change, weight decreases a tiny amount, lipids don't change. But if you look at their metabolites, there's so much going on. And something magic seems to happen, exactly when they terminate diapause.' The most significant magical event: a spike in the amino acid alanine.

Although they don't fully understand alanine's role, Lehmann and his collaborators believe it is key in the termination of diapause. The group is playing with experimentally adjusting the length of the alanine peak in the lab, and looking at differences in alanine peak timing and length in wild populations. Lehmann explains, 'we have this high dynamism that warrants further explanation.' Other studies have shown chemical peaks during diapause that are not well understood. Lehmann feels the correlation between alanine and diapause termination is a strong first step, but there's much more to do. More secrets to unlock in the spirited sleeping butterfly goo.

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