New clues about how carbon dioxide affects bumble bee reproduction

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While a beekeeper puffing clouds of carbon dioxide into a hive to calm the insects is a familiar image to many, less is known about its other effects on bees. A recent study revealed clues about how the chemical

compound affects bee physiology, including reproduction.

The research team, led by an entomologist in Penn State's College of Agricultural Sciences, set out to disentangle how carbon dioxide seems to bypass diapause, a phase similar to hibernation during which bees sleep over the winter, to trigger the reproductive process in bumblebee queens.

The researchers found that carbon dioxide first induced a change in metabolism, which then triggered secondary effects on reproduction. The findings, recently published in *Insect Biochemistry and Molecular Biology*, were contrary to previous hypotheses.

"Previously, it was believed that CO_2 directly affected reproduction, but this study is some of the first evidence showing this is likely not the case," said Etya Amsalem, associate professor of entomology. "We found that CO_2 changes the way macronutrients are stored and reallocated in the body. The fact that the reproductive process is then kickstarted is just an artifact of these processes."

According to the researchers, carbon dioxide is commonly used by beekeepers and researchers to sedate bees and other insects. But in addition to inducing a calming effect, carbon dioxide also can trigger a range of other physiological responses.

For example, while bumblebee queens typically enter diapause through the winter months before starting a new colony in the spring, bee farmers and researchers may use carbon dioxide to trigger the queens' reproductive process earlier than it would happen on its own.

Amsalem said there were multiple reasons she and the other researchers wanted to conduct the study. First, because carbon dioxide is so widely used, she said it's important to understand its mode of action in insects,

and particularly in bumblebees where it is a useful tool for commercial apiaries to produce colonies for pollination year round. But second was to better understand how using carbon dioxide also could influence the results of research.

"We know that carbon dioxide can have many effects, including on behavior," Amsalem said. "So, if you're doing a study on the effects of a specific manipulation on bee behavior and are also using CO_2 as an anesthetic, what are you really studying—the effect of your manipulation or the effect of the CO_2?"

For the study, the researchers performed two phases of experiments. In the first, the researchers wanted a better baseline understanding of the physiological effects of carbon dioxide on the bumblebee queens.

The researchers split the bees into two groups: one that remained untreated and another that was treated with carbon dioxide. The team then examined the bees at three timepoints: right after the treatment and then three and 10 days later. At each timepoint, the researchers examined the ovaries and measured macronutrient concentrations in several tissues, which gave clues about changes in metabolic function over time.

"The queens treated with CO_2 exhibited higher levels of ovarian activation than untreated ones," Amsalem said. "Treated queens also experienced a shift in macronutrient allocation, with fewer lipids in the organ known as the 'fat body' and greater glycogen [a stored form of glucose] and protein in their ovaries."

With this baseline information, the researchers then wanted to tease apart how carbon dioxide affected both metabolism and reproduction. They used carbon dioxide to treat two additional groups of queens: one group that had their ovaries removed, and another that was treated with a

juvenile hormone antagonist.

The researchers explained that this antagonist reduces the levels of juvenile hormone, which regulates reproduction and accelerates metabolism, in bumble bees. The scientists suspected that this hormone is important to the way carbon dioxide could affect insects' physiology.

This time, they found that the bees that had their ovaries removed experienced a similar change in macronutrients to bees in the control group that still had their ovaries. The researchers said this suggested that carbon dioxide affects the metabolism first, since bees without their reproductive organs still experienced the same effect.

Additionally, bees treated with the juvenile hormone inhibitor didn't exhibit these metabolic effects, which the researchers said confirms the role of this hormone in mediating the effects of carbon dioxide.

Amsalem said the findings are important for understanding not just how carbon dioxide affects bumblebees, but all insects.

"CO_2 has many different impacts on insects, so as scientists we're trying to find an effect they all have in common so we can find the precise process or mechanism that is creating these effects," she said. "For example, it positively affects reproduction in bees but might inhibit it in other insects. It's not consistent in the way it affects reproduction. But we think it is consistent in the way it affects metabolism."

Katherine Barie, former graduate student in the College of Agricultural Sciences, and Eran Levin, researcher at Tel Aviv University, also participated in this work.

More information: Katherine Barie et al, CO2 narcosis induces a metabolic shift mediated via juvenile hormone in Bombus impatiens

gynes, *Insect Biochemistry and Molecular Biology* (2022). DOI: 10.1016/j.ibmb.2022.103831