Monarch butterflies rely on temperature-sensitive internal timer while overwintering

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Monarch butterflies at an overwintering site in central Mexico. Credit: D. André Green

The fact that millions of North American monarch butterflies fly thousands of miles each fall and somehow manage to find the same overwintering sites in central Mexican forests and along the California coast, year after year, is pretty mind-blowing.

Once they get there, monarchs spend several months in diapause, a hormonally controlled state of dormancy that aids winter survival. Though diapause is not as obviously impressive as the celebrated annual migrations, it holds mysteries that have perplexed scientists who study biological timing.

Weeks before warming temperatures and longer days signal to the monarchs that it's time to mate and begin spring's northward migration, an internal timer goes off like an alarm clock to rouse the insects, telling them it's time to end diapause and prepare for the critical upcoming events.

Studies in other organisms have shown that cold temperatures can influence the diapause-termination timer, and University of Michigan biologist D. André Green suspected the same is true for monarchs. His study at monarch overwintering sites in central California confirmed it, and his gene expression analyses help explain how cold temperature speeds up that internal timer.

"These results are particularly interesting because they address a counterintuitive result: How does cold temperature, which normally slows down an organism's metabolism and development, speed up diapause? This work is one of the first to provide insights into this question," said Green, a President's Postdoctoral Fellow in the U-M Department of Ecology and Evolutionary Biology who began the work while at the University of Chicago.

The findings have important implications for North America's monarchs—whose populations have declined steadily for decades at the overwintering sites—as the climate changes, Green and co-author

Marcus Kronforst of the University of Chicago wrote in a *Molecular Ecology* study scheduled for publication July 24.

"Understanding how diapause dynamics are affected by environmental and anthropogenic factors at their overwintering sites may be critical for understanding North American monarch population decline and guiding future conservation efforts, a point highlighted by the record low number of monarchs recorded in the western North American monarch population in 2018," Green and Kronforst wrote.

The findings also suggest that monarchs will act as an important sentinel species for monitoring environmental change and disturbance at overwintering sites. If diapause ends too early, monarchs may lose some of the protective time the dormancy period provides.

Green's study involved capturing female monarch butterflies at overwintering sites in central California in November 2015, after they entered diapause. The live insects were brought back to the Chicago lab.

In an environmental chamber there, the butterflies were exposed to temperatures and day lengths approximating November in central California: 10 hours of light at 63 degrees Fahrenheit, followed by 14 hours of darkness at 50 degrees.

In December and again in January, Green's team returned to the same overwintering sites, live-captured additional female monarchs and shipped them to the lab. In the wild, those winter-caught butterflies also experienced short days, along with nighttime temperatures that dipped below 50 degrees.

Green then compared the reproductive maturity of the different groups by counting the number of eggs in each female. An abundance of mature eggs is an indication that the female has terminated diapause, while a paucity of mature eggs indicates that she is still in diapause.

"The monarchs collected from the wild in December showed increased reproductive development compared to the monarchs that had been in the laboratory since November," Green said. "This indicated that an environmental condition in the wild—cold temperature—sped up the timer."

As part of the same study, Green also analyzed gene expression in the different groups of monarchs to understand how the internal timer works. Results suggest that transient markings on histones—proteins around which DNA winds and that control gene expression—may act as a timing mechanism.

The results also show that calcium signaling in the butterfly's head is key, potentially linking the accumulation of cryoprotectants during cold weather to the internal timer.

The research was supported by the National Science Foundation, U.S. Fish and Wildlife Service, and National Institutes of Health. Wild monarchs were collected on private property near Pismo Beach, California, with permission of the landowners.

Green is currently working on a separate study of monarch migration at a study site in U-M's Matthaei Botanical Gardens.

More information: Delbert A. Green et al. Monarch butterflies use an environmentally sensitive, internal timer to control overwintering dynamics, *Molecular Ecology* (2019). DOI: 10.1111/mec.15178

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