

# Pathway found that lets mosquitoes fatten up, slow down for winter

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Two genes that help insulin regulate mosquitoes' growth have been identified as key contributors to how the insects enter a dormant state to survive winter's cold. The research finding broadens the understanding of the mosquito life cycle and appears to shed some light on how other insects and invertebrate species weather the winter months.

The shorter days of autumn trigger certain species of mosquitoes into diapause, a hibernation-like state of arrested development that allows them to survive through the winter. But this new research has determined that a hormonal response is behind the mosquito's ability to store up extra fat and halt reproductive activity in preparation for its months-long dormancy, said David Denlinger, senior author of the study and professor of entomology at Ohio State University.

The research appears online this week in the *Proceedings of the National Academy of Sciences*.

Denlinger's lab is working with *Culex pipiens*, a common mosquito in the United States and the species that carries the West Nile virus in North America. He and colleagues have identified several genes in this mosquito that function within the insulin signaling pathway, the mechanism necessary for diapause to begin. However, they focused on two genes that appear to have the most power in regulating the insect's transition into a dormant state.

“We're trying to decipher this whole pathway – how the shortening days

signal the hormonal shifts that tell mosquitoes to halt development,” Denlinger said.

By disrupting the functions of these two genes, the scientists were able to both mimic hormonal circumstances that initiate diapause as well as disrupt fat retention during diapause – pointing to a separate function for each gene.

While the point at this stage is fully understanding the intricacies of this life cycle, the findings could someday lead to methods to manipulate mosquito populations so they never emerge from their dormant cycles.

“This winter survival is an absolutely critical phase. Most insects cannot survive through the winter unless they go into a dormant state,” Denlinger said. “So we’re trying to understand how this dormant state is regulated. There is a chance it could be manipulated and the mosquito wouldn’t be able to survive the winter. Right now, we’re focused on the mechanism at the physiological and molecular level. But it does have that kind of potential for application in the future.”

The two genes under study are the insulin receptor, which binds to insulin to initiate its action, and a gene known as FOXO, or forkhead transcription factor, which is typically suppressed in the presence of insulin. The hormone insulin regulates blood sugar and body fat storage.

In this species, only females survive the winter. Males and females mate before the onset of winter. Females refuse to take a blood meal, and instead feed only on sugar. Their ovaries stop working, so their eggs will not mature. And they accumulate plenty of fat to sustain them over the winter.

Denlinger’s lab is raising colonies of this species of mosquito in two different artificial day-length environments to either impose a dormancy

cycle or to prevent their shift to diapause. Some mosquitoes are reared under short-day conditions, with 12 hours of light and 12 hours of darkness, and others are reared with long days – 15 hours of light and nine hours of darkness.

Denlinger and study co-author Cheolho Sim, a postdoctoral researcher in entomology, used the two populations to test the insulin signaling pathway genes' effects on diapause.

In mosquitoes living under long daylight conditions, which were prepared to deposit mature eggs, the scientists used a technique to disrupt the insulin receptor gene from completing its function. This disruption halted reproductive activity in the mosquitoes and caused them to enter a state resembling diapause.

On the other end of the diapause spectrum, the scientists disrupted the function of the FOXO gene in mosquitoes that already had entered a dormant state. Within four days, these mosquitoes stopped retaining accumulated fat, killing more than 80 percent of them within three weeks.

Diapause is critical to the survival of the species, as these mosquitoes hold onto their immature eggs through the winter and wait until spring to develop them to maturity and deposit them. Similarly, the West Nile virus appears to be carried within the female from one season to another. Though newly infected birds also could be responsible for the virus's emergence in a given year, it appears that propagation of the virus may be dependent on these mosquitoes' survival, Denlinger said.

Though there is limited evidence so far, other research has suggested the insulin signaling pathway affects dormancy in the roundworm and fruit fly.

“These are very different organisms with a very different evolutionary history – yet they’re using this common pathway of insulin signaling to regulate their dormancy. So an important aspect of this is the suggestion that this might be a common pathway used by lots of organisms to regulate their dormancy period,” Denlinger said.

Warmer temperatures are identified as the signal that brings mosquitoes out of their dormant state, he said. At this stage, they produce a hormone called juvenile hormone, which implies another shift in the insulin signaling pathway at the end of diapause that enables this hormone production.

Source: Ohio State University

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