How the Monarch Butterfly Became a Poison Pill

"We experimentally went back in evolutionary time to reconstruct an event that happened naturally several times, several million years ago." – A. Agrawal

Monarch butterflies (*Danaus plexippus*), the Bambis of the insect world, are beloved by all. But unlike that famous fawn they are loaded with a deadly toxin acquired from the milkweed plants (*Asclepias spp.*) they consume as caterpillars (for the best milkweed to plant in our region see - https://blog.nwf.org/2015/02/twelve-native-milkweeds-for-monarchs/).

Since insects began eating plants over 400 million years ago, plants have evolved a host of chemicals to deter them (http://hras.org/sw/sw501.html). For milkweed the deterrent is a cardiac glycoside, ouabain, a bitter tasting emetic. Ouabain inhibits an essential physiological process, the sodium-potassium (Na-K) pump. The pump regulates the movement of sodium and potassium ions into and out of all animal cells. This movement sets up a gradient of electrical charge critical for the firing of nerve cells and the contraction of heart muscle cells. Ouabain strongly binds to the pump found in heart muscle cells and 'gums' it up. At high enough doses it can stop a beating heart.

Co-opting the milkweed's poison is an effective survival strategy for the monarch. Any naïve bird that eats a monarch will vomit and subsequently avoid the butterfly's distinct orange and black warning coloration. But how did the monarch become immune to the toxic effects of ouabain? Research published in the October 2, 2019 issue of *Nature* reveals the evolutionary pathway the monarch used to become a poison pill to its predators.

The research team lead by evolutionary biologist Noah Whiteman, University of California, Berkeley, Berkeley, CA, and including Amurag Agrawal, Cornell University, Ithaca, NY, used the gene editing technique, CRISPR, to create three key mutations in the gene that builds the Na-K pump in the fruit fly (*Drosophila melanogastor*). These mutations change the pump structure to prevent it from being clogged with poison and the resulting 'monarch flies' (see the 'monarch fly' on a monarch wing) produce larvae that can happily dine on a diet laced with ouabain and, just like monarchs, carry it in their bodies when they change into adults.

The scientists first looked at naturally-occurring mutations in the pump gene of 21 other insects that eat milkweed like the Queen butterfly (*Danaus gilippus*), the milkweed bug (*Oncopeltus fasciatus*) and even another fruit fly (*Dacus siliqualactis*) that lays its eggs on milkweed seeds, which have little poison. This helped them to predict which of the three mutations showed up first and piece together the evolution of the monarch's resistance to ouabain. Then they tried different combinations, adding them sequentially to *D. melanogaster* via CRISPR, to re-create that pathway.

They found that the path to resistance was a three step process. Fly larvae with the mutation that first arose in the monarch's ancestors exhibited some resistance, but the second increased it 10-fold. Adding the third made them resistant to much higher levels of ouabain (1,000-fold), and yielded adults that could also retain lots of poison in their tissues. Gaining each mutation one at a time allowed monarchs to secure a new food source few could eat and repel the many new predators they encountered as they expanded their range across North America.

The mutations had to occur in a specific order, otherwise they have a downside. "It looks as if the mutations protecting the flies against the toxin create a neurological vulnerability. They needed to get the mutations in the right order," said Whiteman. For example, 'monarch flies' engineered only with the third mutation had severe seizures after being shaken.

The team found that 'monarch flies' with only the first mutation had an altered Na-K pump that afforded some resistance but it too made the flies somewhat prone to seizures. Adding the second mutation changed the pump at another site to create an increased level of resistance and it also eliminated the seizures. Now adding the third mutation produced the greatest resistance without introducing seizures because the second mutation was already in place. "Biologists call this a constrained adaptive walk, where one mutation is followed by another, in a particular order, setting a species, or more than one, on a trajectory to higher fitness," said Whiteman.

"Our study suggests that, although there is a universal ancestor to life on earth, small modifications were critical in specific adaptations. We now have the tools to reconstruct how organisms evolved over millions of years," said Agrawal.

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