

New study advances 'DNA revolution,' tells butterflies' evolutionary history

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By tracing nearly 3,000 genes to the earliest common ancestor of butterflies and moths, University of Florida scientists have created an extensive "Tree of Lepidoptera" in the first study to use large-scale, next-generation DNA sequencing.

Among the study's more surprising findings: Butterflies are more closely related to small moths than to large ones, which completely changes scientists' understanding of how [butterflies](#) evolved. The study also found that some insects once classified as moths are actually butterflies, increasing the number of butterfly species higher than previously thought.

"This project advances biodiversity research by providing an evolutionary foundation for a very diverse group of insects, with nearly 160,000 described species," said Akito Kawahara, lead author and assistant curator of Lepidoptera at the Florida Museum of Natural History on the UF campus. "With a tree, we can now understand how the majority of butterfly and moth species evolved."

Available online and to be published in the August print edition of the *Proceedings of the Royal Society B: Biological Sciences*, the study builds the evolutionary framework for future ecological and genetics research of insects, Kawahara said.

"There is a DNA revolution taking place," Kawahara said. "This is an important time in the history of science when we can use DNA

sequencing on a very large scale."

Kawahara said the yearlong study is one of the first to utilize a massive amount of genetic data to answer questions about the history of butterflies and moths. The analysis reveals monumental discoveries about the lineage of Lepidoptera, including strongly contradicting the traditional placement of butterflies in evolutionary history, Kawahara said.

Using next-generation sequencing, a method used to rapidly process large amounts of DNA, scientists developed an initial sample of 46 species that represent many of the most bio diverse groups of moths and butterflies. They also combined 33 new transcriptomes, a set of RNA molecules, with 13 genomes, both of which hold genetic material for organisms. The researchers identified 2,696 genes by breaking down the DNA down and piecing it back together, Kawahara said.

Daniel Rubinoff, entomologist and director of the University of Hawaii Insect Museum, said the new study will help scientists conclusively pinpoint where butterflies belong in evolutionary history—a question that has long troubled researchers.

"This study adds to a growing body of knowledge by bringing new techniques to the table and conclusively demonstrating the evolutionary relationships of the most popular insects on the planet," Rubinoff said. "The methods are novel and build on previous work. This is clearly the future of deep-level evolutionary research."

The wispy, delicate nature of butterflies and moths is part of their charm, but their soft-bodied larval stages have posed a problem for scientists studying them in the fossil record. In the current study, scientists aimed to better understand an evolutionary history that morphological analysis and the fossil record has fallen short of firmly

establishing, said Jesse Breinholt, co-author and a postdoctoral researcher with the Florida Museum.

"The few Lepidoptera fossils we have are from about 15 million years ago," Breinholt said. "The next step is to create a dated [evolutionary history](#) for the group, from the earliest ancestors to present day."

Previous research based on anatomical features hypothesized that butterflies are close relatives of large moths, but the new tree suggests butterflies are more closely related to small (micro) moths, Kawahara said. The study also suggests butterflies are the ancestral group to the tens of thousands of moth species on the planet, and the Hedyliidae family, commonly known as American butterfly-moths, were dismissed as moths and found to be true butterflies.

The tree also provides a baseline to test whether diurnal, or daytime, activity, a common butterfly trait, evolved much earlier than scientists previously believed, possibly at a time when bats' spread across the planet, as a means of escaping these and other nocturnal predators, Kawahara said.

Future research will investigate the causes of evolutionary transitions, such as diurnal activity, across Lepidoptera. Breinholt said although the new tree clarifies our understanding of butterfly and moth relationships, many lineages still need to be examined.

"I hope this is a starting point for larger studies that account for the great diversity of Lepidoptera," Breinholt said.

Provided by University of Florida

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