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DNA Bar Coding Uncovers Secrets of Costa Rican Butterfly

By NICHOLAS WADE

In one of the first uses of DNA bar coding, a new technique for cataloging the planet's species, researchers have uncovered an unexpected richness in the complexity of nature. A long-known butterfly has turned out to be not a single species but 10 different species that live in overlapping territories without interbreeding.

The butterfly, a skipper called *Astraptes fulgerator*, was first described in 1775 and ranges from Texas to northern Argentina. It has long been considered a single species. But Dr. Daniel Janzen, an ecologist at the University of Pennsylvania, began to have doubts.

One of his lifetime goals is to identify every species of caterpillar in a forested area of Costa Rica known as the Area de Conservación Guanacaste. This requires finding a caterpillar and letting it grow into an adult, since most species so far have been identified from the adult form alone.

In the course of 25 years, Dr. Janzen and his Costa Rican colleagues have raised some 2,500 caterpillars of the *Astraptes* skipper. The adults looked almost identical, suggesting that possibly they included several different species, but Dr. Janzen could not come up with any reliable criterion for dividing them into distinct groups. Then he heard of the DNA bar coding technique being advanced by Dr. Paul Hebert, a zoologist at the University of Guelph in Ontario, as a means of inventorying the world's species.

The technique depends on decoding the DNA units of just a single gene, but critics argued that it would not yield a clear separation between different species. Dr. Janzen suspected that his skippers would be perfect for testing the technique's power. He tore off one leg from each of his preserved adult butterflies and sent the severed limbs to Dr. Hebert for analysis.

On the basis of the test gene, the skipper collection fell into 10 separate clusters, according to a report by Dr. Janzen, Dr. Hebert and their colleagues in today's Proceedings of the National Academy of Sciences. The researchers conclude that these are 10 different species because significant differences in DNA sequence imply long evolutionary separation. And the 10 clusters correlated with a fact Dr. Janzen had already observed, that the caterpillars of each feed on different species of plant and have a strikingly different appearances.



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The tropical skipper butterfly is actually 10 different species. Adults from all species look almost identical, but their caterpillars, above, vary significantly.

Why should the adult skippers be almost identical but the caterpillars different? Dr. Janzen said some selective pressure must be forcing the adults to stay the same. His guess is that the skippers' ostentatious color scheme, which includes an iridescent blue splash across the thorax and upper wings, is a kind of mimicry that deters pursuit by birds, a major predator.

The reason is that some butterflies with the blue splash can put on enormous bursts of speed, so that birds do not even try to chase them. Other butterflies have evolved to look like the speedy ones so that birds figure chasing them is a waste of energy. They don't need to be fast, they just need to look fast. "Once you've got the bird into not bothering with bright blue, it doesn't matter how fast you are," Dr. Janzen said. "The caterpillars are living in another world; they are not part of this mimicry ring. So they diverge and the adults stay the same."

Judging by the rate of change in DNA, Dr. Janzen estimates the 10 skipper species started to diverge about four million years ago, probably driven by a shift in feeding preferences.

"This study has altered our view of a 'species' that has been known to science for more than two centuries," revealing "a new layer of biological complexity that needs exploration," the researchers write.

The study is also positive news for DNA bar coding, an ambitious proposal for identifying the world's species genetically instead of by traditional taxonomy. Taxonomists identify species by features in the whole animal. But there are not enough taxonomists to go around, and their highly specialist knowledge is not easily accessible to others.

The goal of DNA bar coding is to enable any researcher, from a field biologist to a museum curator, to tap into the world's heritage of taxonomical knowledge through analysis of the test gene, a stretch of 645 DNA units called the cytochrome c oxidase subunit I gene. The gene is part of the mitochondrial DNA, which lasts far longer than the DNA of the nucleus and is often preserved in dried museum specimens. This means that natural history museums around the world should be able to generate DNA bar codes for many items in their collections, providing a reference database.

Initial field tests of DNA bar coding, like Dr. Janzen's, are showing the value of the technique. "The bar coding is a tremendous additional tool," Dr. Janzen said. "I can predict that this thing is going to go explosive."

For his self-set task of cataloging every caterpillar in the Costa Rican forest, it could be a godsend. Over 25 years he has identified 6,900 but he reckons he has at least 2,000 more to go.