

Scientists use CRISPR to tweak butterfly wing color, change wing scale surface structure

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The African butterfly *Bicyclus anynana* wildtype form (left) and with mutations in the melanin pathway, in particular in the gene *yellow* (right). Credit: William H. Piel and Ant3nia Monteiro

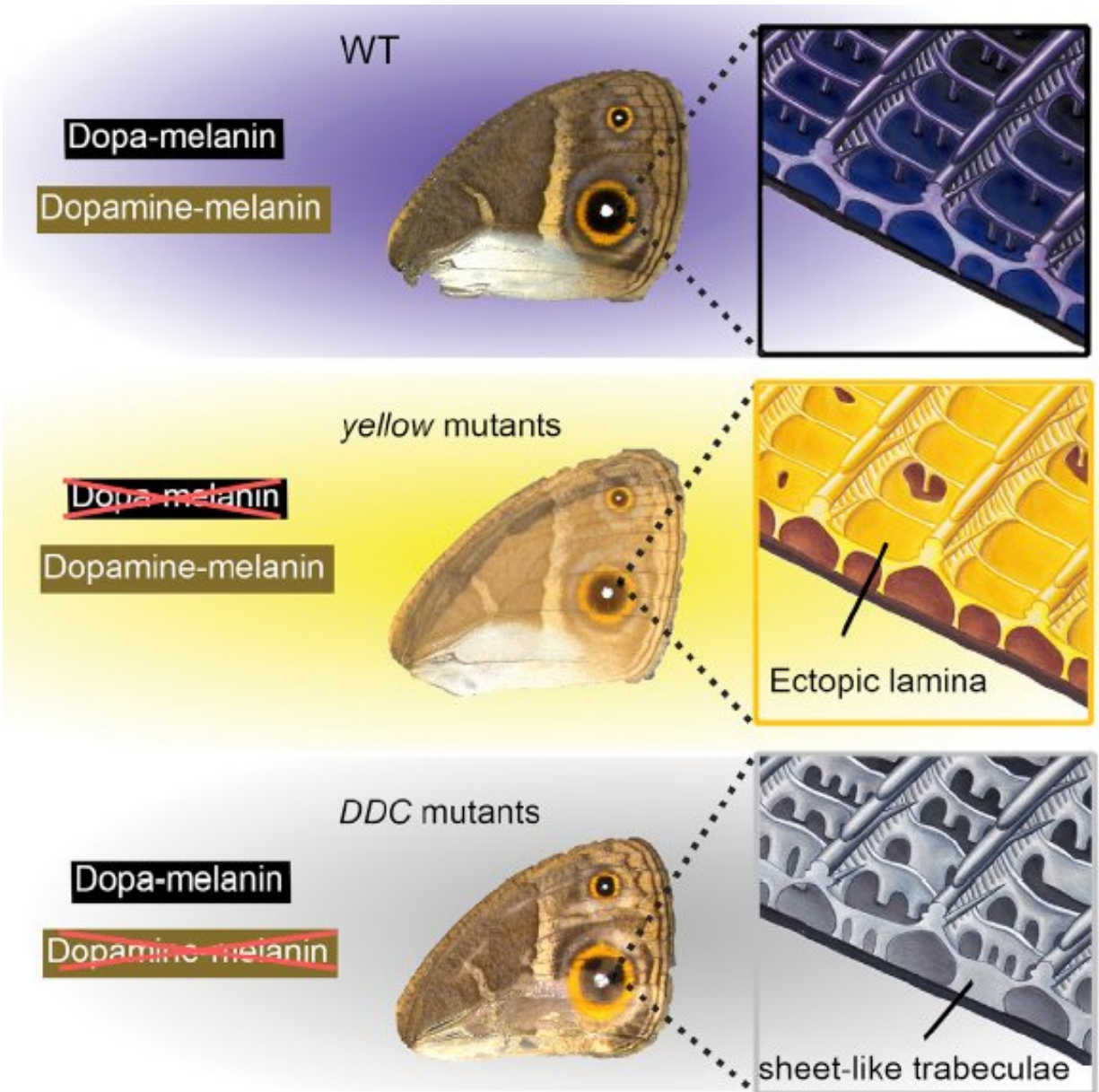
Pigments and the fine structure of butterfly wing scales work together to generate a mosaic of colors and patterns that help the insect camouflage or attract mates. On July 3, in the journal *Cell Reports*, scientists show how it only takes a few pigment genes to modify both the color and the morphology of wing scales. The researchers used CRISPR/Cas9 to tweak wing colors of the squinting bush brown butterfly of East Africa *Bicyclus anynana* and found that it resulted in changes to the scales'

surface structure and rigidity, as well as color. The results show how pigmentation genes have dual roles in the formation of wing scales.

"Our research indicates that the color and structure of [wing](#) scales are intimately related because [pigment](#) molecules also affect the structure of scales," says senior author Ant3nia Monteiro, a biologist at the National University of Singapore's Faculty of Science and Yale-NUS College in Singapore. "Some end products of the melanin pathway, which produces butterfly wing pigments, play a role in both [scale](#) pigmentation and scale morphology."

Postdoctoral fellow Yuji Matsuoka artificially disabled five of *B. anynana*'s pigment genes using CRISPR/Cas9 to better understand how each contributed to the butterfly's wing color. Surprisingly, the mutations altered details of the wing scale structure, too. When a mutation prevented the pigment dopa-melanin from being generated, an extra sheet of chitin, a fibrous component of the exoskeleton, formed horizontally on the upper surface of the wing scale. And when a different mutation blocked the pigment dopamin-melanin, vertical blades of chitin appeared.

"Some butterflies can have vivid hues just by having simple thin films of chitin on their scales that interfere with incoming light to create shades known as structural colors without producing corresponding pigments," says Monteiro. "Light beams reflecting off the top and bottom surfaces of the chitin layer can interfere with each other and accentuate specific colors depending on the thickness of the film, so our results might be interesting in this context."

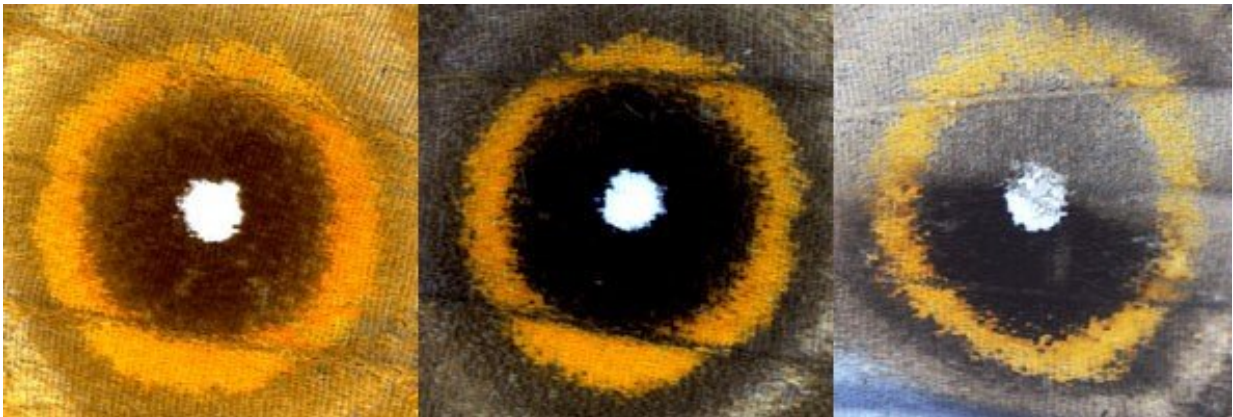


This visual abstract shows the findings of Matsuoka and Monteiro, who discover that deletions of the yellow and DDC melanin genes alter both the color and the morphology of *Bicyclus anynana* wing scales. Credit: Matsuoka & Monteiro/ *Cell Reports*

Wing colors and patterns are critical for butterflies to hide from

predators and signal potential mates; should the mutations introduced in the lab occur naturally, they could jeopardize the insects' survival and reproduction. "Butterfly color has always been described as either pigmentary or structural, but our work identifies the first candidate genes that may constrain the evolution of both of these forms of generating color," says Monteiro.

"The morphology of wing scales is highly diverged; however, the genetic and molecular mechanisms underlying the development of butterfly wing scales have just started to be studied, and melanin products are one of the multiple molecules likely playing a role in this process," says Matsuoka. "Further studies using other butterfly species and other cuticular components will help us understand more about the evolution and development of butterfly wing scales."



Pigmented wing scales in the eyespots of *B. anynana* change color with mutations in the melanin pathway. Yellow mutant (left), wildtype (center), and DDC mutant (right). Credit: Yuji Matsuoka

"If we understand the developmental genetics of [color](#), biotech

companies of the future might be able to generate vivid, brilliant colors via bioengineering, based on butterfly scales, instead of having to nano-manufacture them using metals, which is currently extremely difficult to do," Monteiro says. "These chitin-based colors would be lasting, biodegradable, and environmentally friendly."

More information: *Cell Reports*, Matsuoka & Monteiro: "Melanin pathway genes regulate color and morphology of butterfly wing scales" [www.cell.com/cell-reports/full ... 2211-1247\(18\)30873-8](http://www.cell.com/cell-reports/full ... 2211-1247(18)30873-8)

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