

## Preview

# New molecular insights into butterfly pigmentation

Marcus R. Kronforst<sup>1,\*</sup> and Sofia I. Sheikh<sup>1</sup><sup>1</sup>Department of Ecology & Evolution, The University of Chicago, Chicago, IL 60637, USA\*Correspondence: [mkronforst@uchicago.edu](mailto:mkronforst@uchicago.edu)<https://doi.org/10.1016/j.celrep.2023.112981>

Hanly et al.<sup>1</sup> and Nishida et al.<sup>2</sup> use distinct approaches to provide exceptional lessons regarding the genetic, molecular, morphological, and biochemical bases of butterfly wing pigmentation. These mechanistic insights collectively have important implications for our understanding of phenotypic evolution.

Animal color patterns have a long history of research.<sup>3,4</sup> Butterfly wing patterning, in particular, stands out as an important focal system in the fields of genetics, evolution, and development.<sup>5–7</sup> One might think we have learned almost all there is to know about butterfly wing pigmentation, but two new papers in *Cell Reports*<sup>1,2</sup> detail important discoveries about the underlying mechanisms responsible for the amazing diversity of butterfly color patterns.

Hanly et al.<sup>1</sup> explored the genetic basis of color variation between two closely related “sulphur” butterflies in the genus *Colias*, *C. eurytheme* and *C. philodice* (Figure 1A). Unlike most prior research, which has focused on studying the differences between discrete color pattern morphs, subspecies, or species, Hanly et al. focused on continuous color variation—spanning orange to yellow—between these two hybridizing taxa. The authors used a high-throughput phenotyping method to quantify color variation between species and among experimental crosses. Subsequent quantitative trait locus mapping revealed just two large effect loci, one on chromosome 18 and the other on the Z (sex) chromosome, that together explained 70% of the variation in pigmentation. A separate analysis focused on wing size revealed associations with the Z chromosome and chromosome 2. Ultimately, the authors identified two specific pigmentation candidate genes, *red Malpighian tubules (red)* on chromosome 18 and *bric-a-brac (bab)* on the Z chromosome, which they functionally tested with CRISPR-Cas9 knockouts.

One real stand-out feature of this study is its remarkably comprehensive nature:

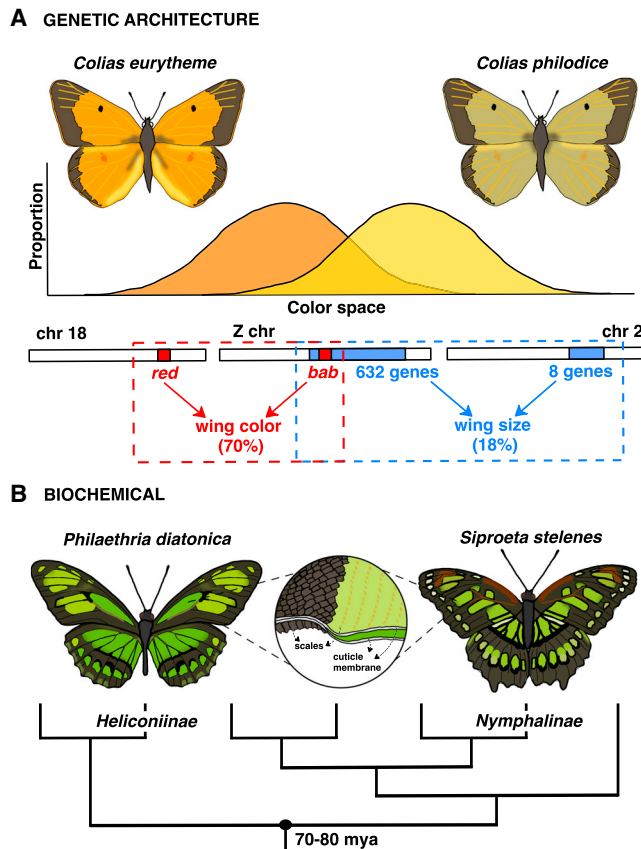
here, the authors quantify color pattern variation, genetically map these differences, identify candidate genes, and ultimately test those genes functionally using CRISPR-Cas9. While this is certainly not the first study to identify specific genes responsible for butterfly color patterning, it is by far the most complete exploration to date, setting a new standard for the field. Conceptually, the work is important because it unlocks the discrete, almost Mendelian, genetic basis of continuous phenotypic variation. While we often think of most phenotypic variation as largely being continuous and the underlying genetic control as being polygenic, butterfly color patterns stand out as strikingly discrete.<sup>8</sup> Hanly et al. show that even when color pattern variation appears more continuous, the underlying genetics may still be quite simple. The Mendelian genetic architecture of mimetic color patterns is often interpreted as a product of natural selection,<sup>9</sup> and Hanly et al.’s results suggest that similar selective pressures may operate on butterfly wing patterns outside the context of mimicry.

In a very different undertaking, Nishida et al.<sup>2</sup> explored the morphological and biochemical bases of vibrant green coloration in two distantly related butterfly species, *Philaethria diatonica* and *Siproeta stelenes* (Figure 1B). Butterfly wing coloration is usually a product of pigments deposited in wing scales or structural properties of the scales themselves. Here, however, the authors discovered an entirely new mechanism of wing pigmentation caused by a green liquid contained within the wing membrane. The authors documented a plethora of living cells in the green portions of the

wing—this is unusual for butterfly wings—that they suggest may be related to green pigmentation. Nishida et al. also identified the molecular basis of green pigmentation, finding that it is a combination of the yellow carotenoid lutein and blue bile pigment. The pigments appear to form a macromolecular assembly with one another and with their own binding proteins *in vivo*. The authors identified these pigments in the butterfly hemolymph, suggesting that the wing pigments are derived from those in the circulatory system.

This discovery of a novel pigmentation mechanism is itself certainly exciting. Beyond the unexpected nature of liquid pigmentation is the even more surprising story of convergent evolution. There are a variety of green butterflies in nature that produce their green hue using well-known properties of scale structure and pigmentation. Yet, Nishida et al. demonstrate that two nymphalid butterfly lineages, which last shared a common ancestor 70–80 million years ago,<sup>10</sup> happened upon the same, unusual liquid pigmentation mechanism to generate strikingly similar green wings. Perhaps this suggests that there is only one way to generate this specific green hue. But why this particular green color? Convergent color patterns often evolve in response to predation,<sup>6</sup> however, the purpose of these vibrant green wings remains a mystery. After many decades of careful study, it is almost unbelievable that we can still, today, learn about a new mechanism of wing pigmentation in butterflies. It is exciting to think about how many other marvelous new findings sit right under our noses awaiting discovery.





**Figure 1. Mechanisms of butterfly wing pigmentation described in two recent studies**

(A) Top: pigment variation within and between two closely related *Colias* species. Bottom: summary of genetic mapping, showing that candidate loci or genomic regions account for 70% and 18% of variation in wing color and size, respectively.

(B) Two distantly related green butterflies have evolved similar wing phenotypes using a novel liquid pigment. Zoom-in shows a dorsal view of the brown/melanic and green regions and a cross-section of the wing, illustrating the green liquid between upper and lower cuticle membrane. Divergence estimate is from Espeland et al.<sup>10</sup>

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### REFERENCES

1. Hanly, J.J., Francescutti, C.M., Loh, L.S., Corning, O.B.W.H., Long, D.J., Nakatani,

M.A., Porter, A.H., and Martin, A. (2023). Genetics of yellow-orange color variation in a pair of sympatric sulfur butterflies. *Cell Rep.* 42, 112820. <https://doi.org/10.1016/j.celrep.2023.112820>.

2. Nishida, K., Adachi, H., Moriyama, M., Futahashi, R., Hanson, P.E., and Kondo, S. (2023).

Butterfly wing color made of pigmented liquid: a novel form of wing coloration. *Cell Rep.* 42, 112917.

- Kronforst, M.R., Barsh, G.S., Kopp, A., Mallet, J., Monteiro, A., Mullen, S.P., Protas, M., Rosenblum, E.B., Schneider, C.J., and Hoekstra, H.E. (2012). Unraveling the thread of nature's tapestry: the genetics of diversity and convergence in animal pigmentation. *Pigment Cell Melanoma Res.* 25, 411–433. <https://doi.org/10.1111/j.1755-148X.2012.01014.x>.
- Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E., Hill, G.E., Jablonski, N.G., Jiggins, C.D., Kelber, A., et al. (2017). The biology of color. *Science* 357, eaan0221. <https://doi.org/10.1126/science.aan0221>.
- Beldade, P., and Brakefield, P.M. (2002). The genetics and evo-devo of butterfly wing patterns. *Nat. Rev. Genet.* 3, 442–452. <https://doi.org/10.1038/nrg818>.
- Deshmukh, R., Baral, S., Gandhimathi, A., Kuvalekar, M., and Kunte, K. (2018). Mimicry in butterflies: co-option and a bag of magnificent developmental genetic tricks. *WIREs Dev. Biol.* 7, e291. <https://doi.org/10.1002/wdev.291>.
- Orteu, A., and Jiggins, C.D. (2020). The genomics of coloration provides insights into adaptive evolution. *Nat. Rev. Genet.* 21, 461–475. <https://doi.org/10.1038/s41576-020-0234-z>.
- Kronforst, M.R., and Papa, R. (2015). The functional basis of wing patterning in *Heliconius* butterflies: The molecules behind mimicry. *Genetics* 200, 1–19. <https://doi.org/10.1534/genetics.114.172387>.
- Jiggins, C.D. (2017). What can we learn about adaptation from the wing pattern genetics of *Heliconius* butterflies? In *Diversity and Evolution of Butterfly Wing Patterns: An Integrative Approach*, T. Sekimura and H.F. Nijhout, eds. (Springer), pp. 173–188. [https://doi.org/10.1007/978-981-10-4956-9\\_9](https://doi.org/10.1007/978-981-10-4956-9_9).
- Espeland, M., Breinholt, J., Willmott, K.R., Warren, A.D., Vila, R., Toussaint, E.F.A., Maunsell, S.C., Aduse-Poku, K., Talavera, G., Eastwood, R., et al. (2018). A comprehensive and dated phylogenomic analysis of butterflies. *Curr. Biol.* 28, 770–778.e5. <https://doi.org/10.1016/j.cub.2018.01.061>.