Commentary

Decoding the black essence of *Melianthus* nectar

Nectar, referred to as the 'drink of the gods' in Homer's Iliad, is a central bridge between flowering plants (angiosperms) and pollinators. The consumption of floral nectar and the subsequent transfer of pollen by pollinators are vital to the reproductive success of most angiosperms. The volume and composition of nectar varies widely between species and with the weather, and even between flowers on the same plant. Sugars such as sucrose, glucose, and fructose are the major rewards for pollinators, and their concentration in nectar has been reported to vary from 7% to as much as 70% per volume of nectar (Pacini & Nicolson, 2007). However, the composition of nectar can be complex, including amino acids that serve as a nitrogen source to pollinators, enzymes that maintain the homeostasis of nectar, phenolics and alkaloids that deter nonpollinators and prevent microbial proliferation, and volatile organic compounds and pigments that serve as attractants to pollinators (Liao *et al*., 2021). The latest to join the list of curious nectar compounds is the black pigment of *Melianthus minor* flowers. In an article published in this issue of *New Phytologist*, Magner *et al*. (2023; 2026–2040) reported that ellagic acid, a heterotetracyclic compound that when complexed with ferric cations (Fe(III)), gives the nectar of *M. minor* flowers its characteristic black color. Coincidentally, this iron-ellagate complex is analogous to the iron-gallate and iron-tannate complexes that constitute the black pigment in iron-gall ink, the most common ink in use before the 20th century (Fig. 1).

> *'Magner* et al. *used an elegant multiomics approach and* in vitro *assays to decipher the biosynthetic pathway of ellagic acid, the black essence of the* Melianthus *nectar.'*

Melianthus is a small rosids genus comprising eight species that are native to the elevated grasslands of southern Africa. *Melianthus* is well known for its dark-colored nectar (Fig. 2) and accounts of its floral characteristics date back to the late 1700s. Due to their unique floral shape, nectar color, and large inflorescence, *Melianthus* species have been cultivated in many botanical gardens around the world. Yet, very few biological studies have been conducted on their flowers, in particular their nectaries. The report by Magner *et al*. is the first comprehensive and systematic study on the nectary development and pollination biology of *Melianthus*.

This article is a Commentary on Magner *et al*. (2023), **239**: 2026–2040.

How do *M. minor* flowers control the color change of their nectar, which only turns black after anthesis?

Through proteomic analysis and transcriptomic profiling, Magner *et al*. determined that a nectar peroxidase, with induced expression during anthesis, catalyzes the oxidation of gallic acid into ellagic acid. Only the latter turns black in the presence of Fe(III).

The RNA sequencing profile also revealed other candidates involved in the production of the black nectar. The detailed functions of these candidates have yet to be confirmed. Moreover, the genes behind gallic acid and iron secretion are still unknown. Interestingly, the unconsumed post-anthesis *M. minor* nectar gradually loses its black color (Fig. 2), which potentially provides a mechanism to deter pollinator visitation to post-anthesis flowers. While it is beyond the scope of the current study, it would be interesting to examine the changes in the chemical composition and transcriptome of post-anthesis *Melianthus* nectar and nectary, with the hope of determining the biochemical pathways behind the loss of coloration as well. Future studies identifying the remaining players in the biosynthetic and degradation pathways will offer a more complete picture of black nectar synthesis in *Melianthus*.

What is the pollinator of *Melianthus* and how does the black color of *Melianthus* nectar contribute to pollinator attraction?

Previous observations have documented both insect and bird visitation to *Melianthus* flowers for their nectar. Magner *et al*. took a further step to examine the relevance of its black color to pollinator attraction. They adapted visual modeling, a widely used practice in sensory ecology, to explore the visibility of the black nectar and its contrast to petals and foliage to both birds and bees, allowing them to determine that the black color is indeed highly conspicuous to birds but not to bees. A recent study by the same research group employed a similar visual modeling approach to demonstrate the visibility of the blood-red nectar of *Nesocodon* (Campanulaceae) to geckos (Roy *et al*., 2022). Together, these studies demonstrate the power of such interdisciplinary approaches in bridging our understanding of floral development and plant biotic interaction. In the case of *Melianthus*, it would be exciting to see future field pollination biology studies that directly test the influence of the black nectar color on pollinator behaviors.

What is the evolutionary origin of the black nectar in *Melianthus* and other angiosperms?

All eight species of *Melianthus* have been reported to produce dark-colored nectar, ranging from light gray to black, while their most closely related genus *Bersama* produces clear nectar (Henning, 2003). The evolutionary history of these lineages makes

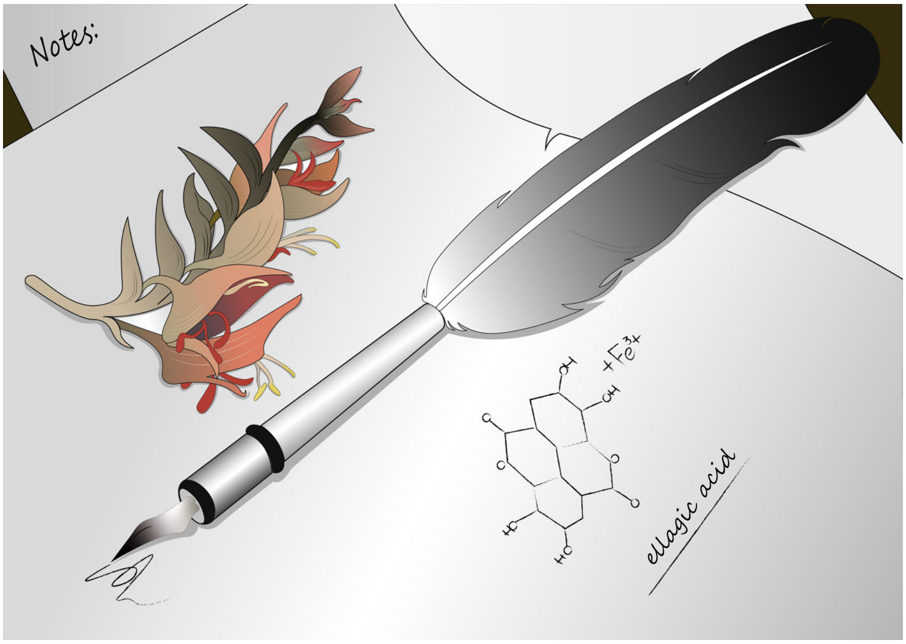

Fig. 1 An illustration by Esmeralda Izquierdo showing the black nectar of *Melianthus* and its analogy to iron gall ink.

Fig. 2 Developmental progression (right to left) of a *Melianthus minor* flower. This figure is kindly provided by Evin Magner.

them a great system to study the evolution of specialized nectar traits and the implication of these traits in pollinator interaction and reproductive success. Future studies investigating the genetic variation of the iron-ellagate complex production pathways in these closely related species will shed further light on the evolution of black nectar. Furthermore, many other floral traits associated with nectar production and pollinator attractions, including petal size, nectar volume, and inflorescence organization, have been shown to be highly variable among *Melianthus* species (Henning, 2003). To what extent is each of these individual traits contributing to specific pollinator attraction? And what kind of synergistic interactions arise between those traits and the peculiar black nectar? Perhaps quantitative trait locus mapping to explain pollinator visitation rate will provide more insights in these aspects.

More broadly, colored nectar has been documented in at least 67 taxa worldwide, and the trait is believed to have independently evolved 15 times at the family level in angiosperms (Hansen *et al*., 2007). Some *Schiedea* (Caryophyllaceae) species also produce black floral nectar. Do any of these species share any process homology in their nectar pigment production, and how are the pathways behind nectar pigment production genetically controlled? From genetic screenings and transcriptomic analysis in various nectaries across angiosperms, dozens of genes and metabolic pathways have been identified to participate in nectar production (Slavkovic *et al*., 2021). However, our knowledge of the genetic architectures underlying nectary development and their variation across taxa is still minimal. Future comparative work investigating the control of colored nectar production in diverse taxa can, therefore, offer a unique perspective on the development, physiology, ecology, and evolution of this critical plant product.

The work by Magner *et al.* provides us with an approach and system to explore these questions and a fresh textbook example of nectary biology studies in the modern era.

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ORCID

Lily Cheung https://orcid.org/0000-0001-8089-7783
Yan Gong https://orcid.org/0000-0003-1329-7096
Elena M. Kramer https://orcid.org/0000-0002-5757-1088

Yan Gong[1]*, Lily Cheung[2]* and Elena M. Kramer[1]

[1]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA;
[2]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA
(*Authors for correspondence: emails yangong@fas.harvard.edu (YG); lily.cheung@gatech.edu (LC))

References

Hansen DM, Olesen JM, Mione T, Johnson SD, Muller CB. 2007. Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait. *Biological Reviews of the Cambridge Philosophical Society* **82**: 83–111.

Henning J. 2003. *Further studies with* Melianthus *L.: a molecular phylogeny, evolutionary patterns of diversification in the genus and pollinator syndromes.* Cape Town, South Africa: University of Cape Town.

Liao IT, Hileman LC, Roy R. 2021. On the horizon for nectar-related research. *American Journal of Botany* **108**: 2326–2330.

Magner ET, Roy R, Freund Saxhaug K, Zambre A, Bruns K, Snell-Rood EC, Hampton M, Hegeman AD, Carter CJ. 2023. Post-secretory synthesis of a natural analog of iron-gall ink in the black nectar of *Melianthus* spp. *New Phytologist* **239**: 2026–2040.

Pacini E, Nicolson SW. 2007. Introduction. In: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and nectar.* Dordrecht, the Netherlands: Springer Netherlands, 1–18.

Roy R, Moreno N, Brockman SA, Kostanecki A, Zambre A, Holl C, Solhaug EM, Minami A, Snell-Rood EC, Hampton M *et al.* 2022. Convergent evolution of a blood-red nectar pigment in vertebrate-pollinated flowers. *Proceedings of the National Academy of Sciences, USA* **119**: e2114420119.

Slavkovic F, Dogimont C, Morin H, Boualem A, Bendahmane A. 2021. The genetic control of nectary development. *Trends in Plant Science* **26**: 260–271.

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