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Authors

Liao, Irene T
Hileman, Lena C
Roy, Rahul

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ON THE NATURE OF THINGS: ESSAYS

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On the horizon for nectar-related research

Irene T. Liao[1] | Lena C. Hileman[2] | Rahul Roy[3]

[1]Department of Molecular, Cell, and Developmental Biology, University of California—Los Angeles, 610 Charles E Young Dr East, Los Angeles, CA 90095, USA

[2]Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Ave., Lawrence, KS 66045, USA

[3]Department of Biology, St. Catherine University, 2004 Randolph Avenue, St. Paul, MN 55105, USA

Correspondence
Lena C. Hileman, Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Ave., Lawrence, KS 66045, USA.
Email: lhileman@ku.edu

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The relationship between flowering plant nectars and interacting animals is fundamental to plant fitness, shaping macroevolutionary and macroecological patterns of trait and community diversity. Researchers are addressing broad-ranging questions that encompass community and microbiome assembly, evolutionary patterns and processes, nectar biochemistry, and nectary and nectar spur development. In light of the interdisciplinary nature of nectar-related research, here we focus on questions that are *on the horizon*—where the tools and models are emerging and where integration and collaboration across research specialization is likely to facilitate discovery.

NECTAR COMPOSITION

The complex microbial (Figure 1C) and chemical (Figure 1F) milieu of nectar with sugars, proteins, and other diverse metabolites, including ions, is important for mediating ecological interactions ranging from plant–pollinator and plant–defender interactions (Figure 1A, B) to plant–microbiome dynamics. Discovering how different species produce unique nectar chemical diversity is key to fully understanding how nectar functions at the ecological nexus of biotic interactions. Seminal work on the relationship between sugar composition and pollinator preference (e.g., Baker and Baker, 1983) provides a springboard for continued investigation into sugars, other metabolites, and the nectar microbiome. For example, the integration of transcriptomic, biochemical, and metabolomic analyses led to the discovery of pathways that produce a novel blood-red pigment in *Nesocodon mauritianus* nectar. This red nectar is a crucial visual cue for geckos, the likely primary pollinators of *N. mauritianus* (Roy et al., 2021 [Preprint]). Specialized nectar metabolites such as nicotine, caffeine, and gelsemine have been shown to play crucial roles in modifying pollinator visitation (reviewed by Stevenson et al., 2017), while the nectar microbiome also affects pollinator attraction (e.g., Colda et al., 2021).

On the horizon for nectar composition is discovering the potential diversity of uncharacterized nectar metabolites. In many cases, these metabolites can be discovered directly, but transcriptomic and proteomic approaches can also lead to metabolite profile predictions. Deep application of ionomic, metabolomic (Figure 1I, J), transcriptomic (Figure 1G, H), and proteomic approaches in a diversity of plants are likely to uncover novel nectar components. Likewise, deep sequencing of nectar microbiomes will inform both their complexity and patterns of community assembly. Coupling these "-omic" results with field studies will lead to a more complete characterization of the links between nectar components and emergent plant–animal and plant–microbial interactions.

NECTARY CELL BIOLOGY AND PHYSIOLOGY

Complex physiological and cellular processes with well-orchestrated molecular and biochemical events lead to nectar production, nectar secretion, and possibly nectar reabsorption (reviewed by Roy et al., 2017). Nectaries can range from completely unstructured to spatially organized tissues with distinct functions, such as epidermal cells for secretion and parenchymal cells for starch storage and nectar transport (Nicolson et al., 2007). In addition, nectaries may undergo temporal processes, such as starch accumulation in early development and starch breakdown to produce sugars for secretion in later development. Nectar secretion mechanisms also vary across species, ranging from

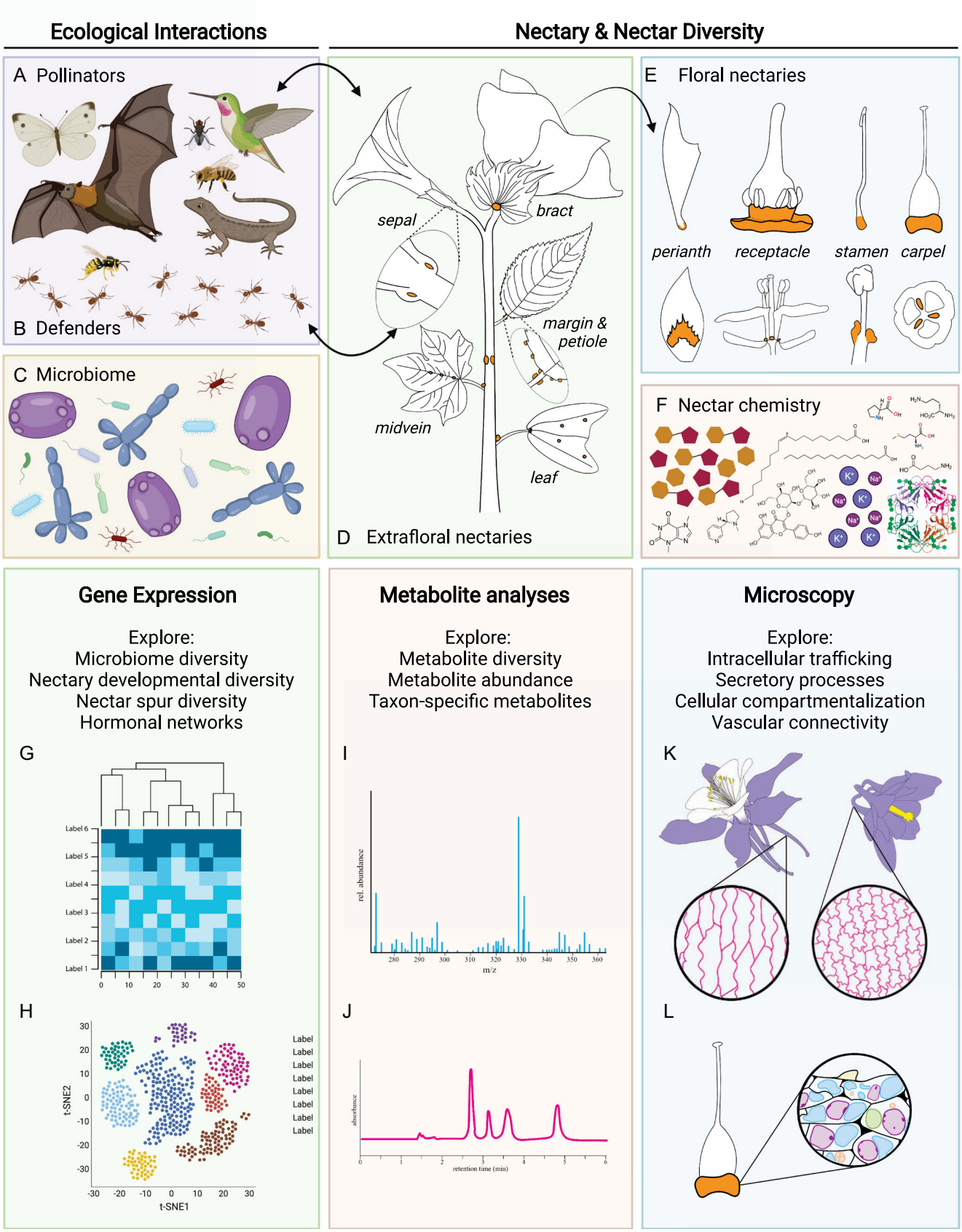

FIGURE 1 (See caption on next page)

eccrine (membrane transporter-based secretion) to merocrine (vesicle-packaged based secretion) to holocrine (epidermal rupture-based secretion). Fully understanding nectar production and secretion requires characterizing the spatial and temporal processes inside nectaries at the tissue and cellular level across diverse species.

A number of research avenues that are *on the horizon* will provide a framework for identifying the cellular and physiological dynamics of nectar production. A key direction will be visualizing sugar and metabolite processing and transport during active nectar production and secretion using techniques such as microscopic imaging coupled with cell permeable dyes and molecular probes (e.g., Solhaug et al., 2021) (Figure 1L). Along similar lines, experiments utilizing carbon/nitrogen isotopes will shed light on how amino acids and sugars are trafficked into nectar, whether they are synthesized in nectaries de novo, derived from vasculature sources, or a mixture of both (e.g., Solhaug et al., 2021). Plant hormones such as auxin, gibberellins, and jasmonic acid play critical roles in nectar production (reviewed by Roy et al., 2017). Therefore, expanded analysis of hormone networks (e.g., Figure 1G, H) will be key in understanding how metabolism, transport, and secretion of nectar metabolites are regulated. Lastly, single cell RNA sequencing technology (Figure 1H) is poised to provide the ground plan for our understanding of how cellular functions are partitioned across nectary cell and tissue types.

COMPARATIVE NECTARY DEVELOPMENT

Floral and extrafloral nectaries (Figure 1D, E) have evolved independently multiple times during flowering plant diversification (e.g., Nicolson et al., 2007; Weber and Keeler, 2013). Nectaries can be associated with any plant structure except the root; they have diverse morphologies, and nectar can be secreted through modified trichomes, modified stomata, and even through cell rupture (Nicolson et al., 2007). Therefore, the uniting feature of a nectary is simply its nectar-secreting function. Given the association of nectaries with diverse plant structures, yet exhibiting an underlying nectar secreting function, a key question is whether similar genetic programs underlie nectary development. Addressing this question requires a comparative approach.

A number of strong candidates for regulating nectary initiation have been discovered. *CRABS CLAW* (*CRC*), a YABBY-like transcription factor involved in gynoecium development, is also critical for the initiation of floral nectary development in *Arabidopsis* (Baum et al., 2001) and *Petunia* (Lee et al., 2005). Notably, *CRC* expression is strongly associated with diverse floral and extrafloral nectaries in core eudicots, but not in other flowering plant lineages (Lee et al., 2005). In the early-diverging eudicot lineage *Aquilegia*, nectaries are found at the tips of petal nectar spurs, and the gene *STYLISH* (also involved in gynoecium development), but not *CRC*, was found to have a key role (Min et al., 2019). Recently, GoNe, an APETELA2/ethylene-responsive element-binding transcription factor, was found to be necessary for both floral and extrafloral nectary development in cotton independent of *CRC* (Pei et al., 2021). These initial insights suggest that diverse initiators of nectary development may have evolved in divergent flowering plant lineages.

On the horizon for nectary development is determining whether the *CRC*-dependent program is globally recruited for nectary initiation across eudicots and whether analogous programs (non-*CRC* dependent) are similarly co-opted for nectary initiation in monocots and other non-core eudicot lineages. Addressing these hypotheses requires transcriptomic profiling (e.g., Figure 1G and H) and functional genetics in a phylogenetically informed framework for the discovery of nectary-associated genes and downstream targets. Floral and extrafloral nectary transcriptomic data from across flowering plants are quickly being generated and functional genetic approaches developed for many newly emerging flowering plant models. By integrating studies of nectary development within and across taxa, these approaches will uncover the extent to which convergent evolution of nectaries is the result of repeatedly recruited developmental programs.

COMPARATIVE NECTAR SPUR DEVELOPMENT

Many nectar-rewarding species accumulate floral nectar in spurs which, in turn, are critical to many flower–pollinator interactions. As with nectaries themselves, aspects of nectar spur homology remain complicated—such as initiation from different organ types and underlying molecular programming.

FIGURE 1 Nectar is at the center of flower–organismal–ecological interactions. The importance of nectar is reflected in abundant chemical and microbial diversity as well as nectary structural and functional diversity. Example animal (A) pollinators (hummingbirds, bees, bats, butterflies, moths, flies, geckos) and (B) defenders (ants, wasps) that feed on nectar. (C) Fungi, especially yeast, and bacteria comprise the microbiome in nectar. (D) Whole-plant architecture depicting example locations of extrafloral nectaries (in orange, bold outline). (E) Floral nectaries can be associated with multiple floral organs including the perianth, receptacle, stamen, and carpel. (F) Nectar chemical composition includes sugars, lipids, proteins, ions, amino acids, and phenolics. High-throughput gene expression studies, for example, (G) tissue-specific RNA sequencing and (H) single-cell RNA sequencing, uncover nectary and nectar spur developmental genetic programs. Metabolite analyses, for example, (I) mass spectrometry and (J) high-pressure liquid chromatography, provide insights into nectar chemical diversity. Microscopy techniques, for example, (K) light or fluorescence microscopy and (L) electron microscopy (EM; example transmission EM shown, but also includes scanning EM), reveal cellular and subcellular organization and dynamics of nectary and spur development and nectar production and secretory processes. Elements of the figure were drawn and created with BioRender.com

Regardless, programming of spur development usually requires initiation of a cell proliferation zone on a laminar surface (e.g., *Linaria* petal spurs and *Delphinium* sepal spurs). Indeed, recent work by Ballerini and colleagues (2020) showed that a cell-proliferation regulator underlies presence/absence of spur formation in *Aquilegia*. Other studies suggest possibly divergent developmental pathways for spur initiation involving foci of meristem identity (*Linaria*; Box et al., 2011) or auxin signaling (*Aquilegia*; Yant et al., 2015). Additionally, interspecific variation in spur length and shape can be controlled by divergent mechanisms of cell proliferation and cell expansion, leading to final spur morphologies (Puzey et al., 2012; Cullen et al., 2018).

On the horizon for nectar spur development is discovering the extent to which convergent origins of spur formation and spur elongation employ similar developmental mechanisms. Integration of highly paralleled transcriptomic (e.g., Figure 1G, H) and forward-genetic mutagenesis approaches will lead to detailed understanding of spur initiation across multiple model species, and therefore assessment of genetic parallelism. With respect to spur length variation, comparative work addressing the relative role of cell proliferation and expansion processes will help clarify their individual contributions (e.g., Puzey et al., 2012; Cullen et al., 2018) (e.g., Figure 1K). Phytohormone signaling likely underlies these cell-level processes as recent work suggests that auxin, brassinosteriods, and cytokinins broadly affect floral organ size variation (reviewed by Wessinger and Hileman, 2020). Therefore, phytohormones likely play a role in regulating spur elongation. Their potential role, as well as alternative pathways, will be revealed through integration of biochemical, transcriptomic, mutagenesis and QTL studies (e.g., Edwards et al., 2021) among closely related species differing in spur length.

CONCLUSIONS

Parallelism in nectary function and development make for an ideal system to integrate across multiple levels of organization (cellular, physiological, developmental, genetic, phylogenetic, ecological) to address the fundamental question of how nature repeatedly lands on common solutions to ecological problems—in this case, the optimization of plant–animal–microbial interactions. It is clear that the community is eager to collaborate across disciplines to advance our understanding of nectar biology, especially in a comparative framework. We encourage our many colleagues to connect and develop collaborations that address nectar-related questions that are *on the horizon* and beyond.

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AUTHOR CONTRIBUTIONS

I.T.L., L.C.H, and R.R. all contributed equally to conceptualization, drafting, and writing.

ORCID

Irene T. Liao http://orcid.org/0000-0002-2904-4117
Lena C. Hileman http://orcid.org/0000-0001-6116-666X
Rahul Roy http://orcid.org/0000-0002-2241-2976

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