Trends in
Ecology & Evolution

CellPress

Forum

Herbaria unlock tripartite pollination responses to anthropogenic change

Lauren H. Puleo [1,*], Tadashi Fukami [1,2], and Barnabas H. Daru [1,*]

Pollination involves plants, pollinators, and microorganisms, challenging the traditional bipartite understanding. Floral nectar is the primary medium where tripartite plant–pollinator–microbe interactions occur. These interactions face anthropogenic disruptions temporally, geographically, and across diverse taxa. Herbarium specimens can provide untapped historical records to understand their long-term response to anthropogenic change.

Pollination in a changing world

Pollination is an essential process that underlies biodiversity patterns and sustains ecosystems. Animal-mediated pollination has long been viewed as a bipartite interaction between plants and pollinators. However, recent studies have revealed that flower-inhabiting microorganisms influence plant–pollinator mutualisms [1,2], calling for a conceptual expansion of pollination to involve three interacting partners: flowering plants, animal pollinators, and microorganisms [3].

Floral nectar (see Glossary) is the interface where these **tripartite interactions** occur. Many angiosperms produce floral nectar, a trait that facilitates plant–pollinator coevolution and has evolved independently multiple times across plant phylogeny [4]. **Animal pollinators** visit flowers to obtain nectar and pollen to power their metabolic needs while performing pollination by transferring pollen between flowers. During this process, pollinators serve as the mechanism for microbial dispersal across flowers [1]. **Nectar microbial symbionts** modify nectar composition and release volatile organic compounds, which can influence pollinator behavior and plant fertilization success [3]. These nectar-inhabiting microorganisms are specialists thriving in the nectar niche and are conserved with low species richness, typically belonging to the yeast family Metschnikowiaceae and the bacterial genera *Acinetobacter, Neokomagataea*, *Erwinia/Pantoea*, *Pseudomonas*, and *Rosenbergiella* [1,2]. Together, these plants, pollinators, and microorganisms form an intertwined tripartite interaction, whose synchrony has likely been shaped over millions of years of evolution.

However, these interacting species are threatened by environmental change. Modern environmental change has disrupted the temporal alignment of **phenological events** and created geographic mismatches between microbial communities, their host plants, and pollinator dispersers in some regions [5]. These disruptions may cause taxonomic shifts in interactions among plants, pollinators, and microbes, such that species diversity and composition are more altered. In these ways, anthropogenic change can rapidly disrupt plant–pollinator–microbe interactions.

While advancements have been made to characterize how floral nectar shapes pollination interactions in the present day [1,2,6], we currently lack historical data spanning past decades of environmental change, in which **herbarium specimens** have a role to play.

Herbarium specimens can capture changes in tripartite pollination interactions

Herbarium specimens were not originally collected to document ecological

Glossary

Animal pollinators: animal species, such as invertebrates (e.g., bees, butterflies, beetles, and flies) and vertebrates (e.g., birds and small mammals), that provide the service of pollination to flowering plants.
Crystallized nectar: dehydrated nectar from dried herbarium specimen flowers, serving as the preserved medium of pollination interactions. During the drying and pressing phases of creating an herbarium specimen, liquid nectar evaporates and leaves behind a crystallized solute residue containing the nectar's organic compounds, such as sugars, and dead microorganisms.
Environmental DNA (eDNA) metabarcoding: a DNA sequencing methodology that uses genetic material left behind by organisms in their environment to simultaneously identify many taxa that have inhabited that environment or interacted with a specific plant.
Floral nectar: a viscous substance from plant flowers, rich in organic compounds, including sugars (fructose, glucose, and sucrose), amino acids, lipids, and secondary metabolites. Floral nectar is an attractive and rewarding floral trait that fulfils the dietary requirements of visiting animal pollinators and provides the physical and nutritional medium for microbial growth, especially that of yeasts and bacteria.
Generalized and specialized pollination systems: a generalized pollination system describes a plant that has a mutualistic relationship with a broad range of pollinators. A specialized pollination system describes a plant that has a mutualistic relationship with a small number of pollinator species. Pollination interactions exist along a continuum from generalized to specialized.
Herbarium specimens: dried and pressed plant samples, originally gathered for taxonomic and systematic purposes, that have been collected globally from 1532 to the present day.
Nectar microbial symbionts: specialized yeasts and bacteria that inhabit floral nectar. When a flower blooms, its nectar is initially sterile until pollinator visitation introduces microorganisms. Subsequent microbial colonization is controlled by priority effects and competition dynamics, with early-arriving species strongly influencing community assembly.
Phenological events: the timing of life-history events.
Phenological synchrony: a temporal window when all three pollination partners are optimally active. During this period, plants flower and produce food and resources, pollinators emerge from hibernation or migration with high energy demands, and microbes rapidly colonize plant flowers.
Pollination syndromes: floral traits, such as corolla tube length, color, and floral nectar sugar composition, that are selected by and attract specific pollinators.
Tripartite interactions: interactions between three different species across trophic levels.

interactions, yet they provide valuable data spanning centuries of plant–pollinator–microbe interactions. Their value lies in the metadata they capture: species identity (taxonomic), collection locality (geographic), and collection date (temporal) (Figure 1). The physical specimen also captures **pollination syndromes** and reproductive organs that underpin pollination biology (Figure 1). Herbarium specimen data contain sampling biases because specimens are typically collected opportunistically, based on accessibility or phenological stage, rather than systematically, which necessitates careful statistical treatment when inferring ecological patterns (Box 1) [8].

Nectar sugar composition is considered a pollination syndrome, with research suggesting that plant flowers exhibit conserved floral nectar sugar compositions, selected by their most frequent pollinator visitors and subsequently modified by microbial inhabitants [6]. Although floral nectar typically desiccates during specimen preparation, the sugars in the resulting **crystallized nectar** can remain stable over time [10], along with other nectar components such as amino acids, lipids, and secondary metabolites [9]. While research has not yet characterized nectar from herbarium specimens, rehydrating crystallized nectar and analyzing its chemical composition using liquid-chromatography–mass-spectrometry, a common approach for fresh nectar, may reveal how this trait, fundamental to plant–pollinator–microbe interactions, has responded to anthropogenic change [2,6,9].

Beyond nectar chemistry, nectar microbial communities serve as indicators of pollination services, with some studies suggesting that floral nectar microbial communities act as fingerprints of pollinator visitation [2]. While herbarium microbial research has advanced due to improvements in DNA extraction and sequencing methodologies, these studies primarily focus on plant–microbe interactions in leaf tissue [8] and roots [11]. Crystallized nectar likely retains dead yeasts and bacteria, and advancements in DNA extraction methods can reveal their compositional shifts over the last century. To capture the full picture of floral nectar microbial diversity, herbarium flowers at mature life stages should be sampled when microbial communities have reached a late successional stage. In

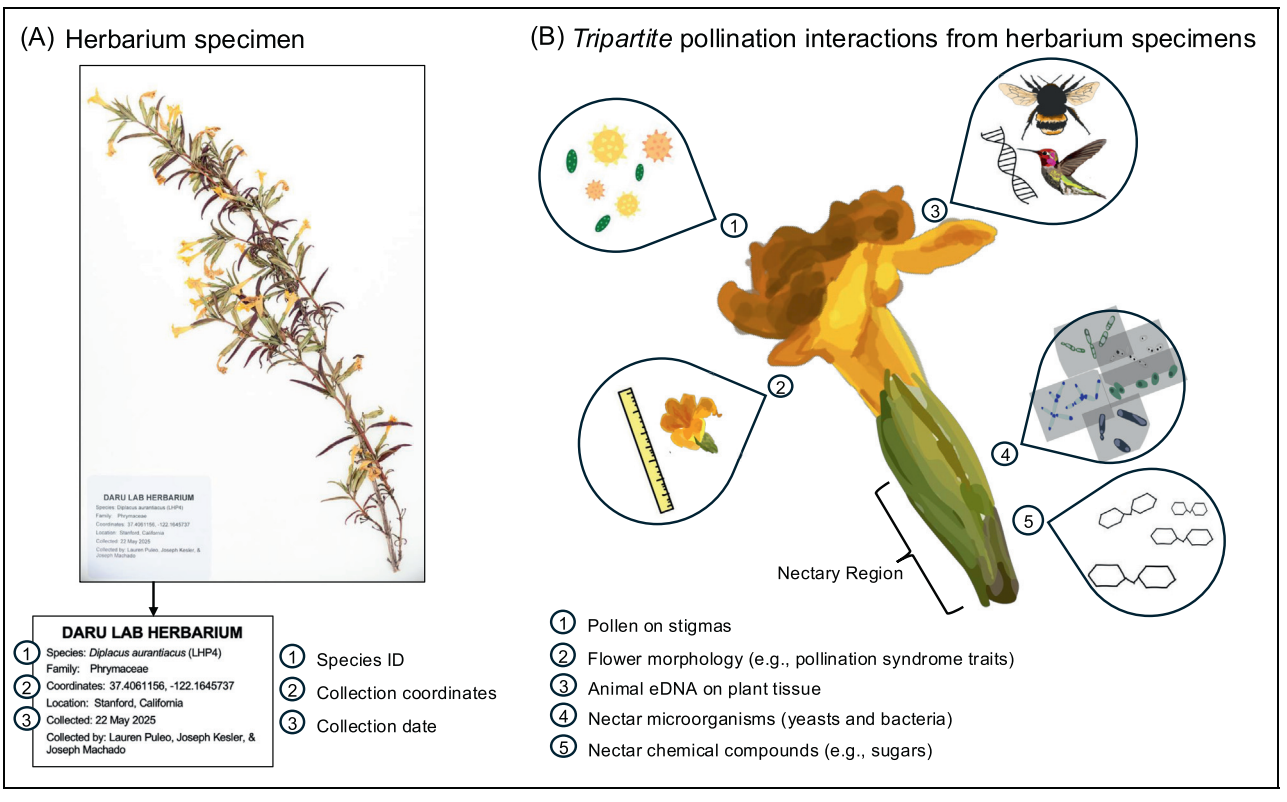

Figure 1. Expanding herbarium research to capture tripartite pollination interactions. (A) Herbarium labels provide species identification, collection dates, and geographic metadata. (B) Integrating established methods (analyzing pollen on stigmas and measuring floral morphology) with emerging approaches (animal eDNA and crystallized nectar analyses) can enable the inference of plant–pollinator–microbe interactions. Image courtesy of Callie Chappell.

some species, flower age can be estimated from morphological characteristics observable in herbarium specimens. Advances in this field will further open the door to examining other floral microbes, including pollen-borne microbes.

Further, herbarium specimens are finding new applications in sequencing techniques, such as **environmental DNA (eDNA) metabarcoding**. The eDNA recovered from herbarium specimens has enabled the identification of historical animal visitors [12] and could be expanded to identify specific pollinator visitors. Likewise, advances in computer vision models can enable the automated extraction of morphological floral traits from digitized specimens. For instance, population-level changes in tubular corollas, which are often associated with bird and moth pollination, can be tracked over decades to detect morphological shifts in response to changing pollinator communities. Such emerging methods could be paired with greenhouse experiments and present-day collections, along with existing approaches (e.g., pollen [13]), to validate the past patterns and interactions inferred from herbarium specimens (Figure 1; Box 1). From here, based on the presence of traits and microorganisms, pollination network structure can be estimated using statistical approaches ([14]). Altogether, herbarium specimens can enable the analysis of pollination interactions and reveal how their synchrony might be disrupted over temporal, geographic, and taxonomic dimensions.

Temporal scope

Phenological synchrony is essential for pollination but is threatened by environmental change [5]. Environmental perturbations can cause phenological shifts and temporal mismatches, where one or more partners are active while others are not [5]. Herbarium data provide unparalleled temporal depth beyond most long-term monitoring efforts and can reveal how modern phenological events deviate from historical baselines and expected evolutionary patterns (Box 1) [7].

Geographic scope

Geographic mismatches can occur among plants, pollinators, and microbes if species respond differently to environmental change. Species distributions are already shifting in response to changing environmental and climatic conditions [5]. For instance, a plant species might experience a westward range shift to track suitable climates, while its specialized pollinator might shift eastward following precipitation patterns, and its symbiotic microbes might shift according to local resource availability [15]. Differential responses to anthropogenic change can, therefore, threaten tripartite pollination relationships. However, it is also possible that the diffuse coevolution that may be underlying these interactions confers increased resilience to change. Herbarium specimens are a unique resource to test these hypotheses.

Herbarium specimens provide the primary data for modeling plant range shifts across regions, and the physical specimens harbor microbial communities in nectar that can be used to model the concurrent range shifts of both plants and microbes. Moreover, herbarium trait analyses and microbial fingerprint signatures can associate plant hosts with local pollinator visitors. Together, herbarium collections are untapped resources for understanding and predicting the geographic shifts of plants, pollinators, and microbes (Box 1).

Taxonomic scope

Environmental change may cause taxonomic mismatches among interacting partners. As temporal and geographic mismatches occur via nonnative introductions, or differences in dispersal rates and range shifts, novel interaction networks may emerge. These networks may arise through host-switching or shifts in

Box 1. How to study tripartite pollination with herbaria?

Reconstructing changes in tripartite pollination interactions

Analyzing taxonomically diverse specimens can reveal shifts in **generalized and specialized pollination systems**, with specialized systems potentially showing greater sensitivity to environmental change. Temporal variation in traits across plant lineages may further reveal how environmental change alters tripartite interactions. Specimens sampled across geographic gradients may reveal spatial variation in traits and interactions (Figure I).

Herbarium specimens collected throughout the flowering season from similar geographic regions may reveal within-season variation, while comparing specimens collected over many years may differentiate short-term seasonal fluctuations from longer-term population-level changes driven by environmental change (Figure I).

Caveats

Herbarium specimens were not originally collected for ecological purposes. Sample size and coverage may be too limited and biased for some questions. Careful statistical treatment of data is essential [7]. Furthermore, surface contamination can interfere with microbial DNA extractions, and biochemical components may degrade over time. However, studies have successfully detected target microorganisms [8] and profiled chemical compounds [9] from herbarium specimens—methods that could be modified for crystallized nectar. Aging effects could also be experimentally controlled by artificially aging herbarium flowers or by comparing geographic gradients of specimens over time to determine whether biochemical information is consistent and preserved.

Targeting nectar microbiomes

Surface contamination is minimized when analyzing microbes expected only in floral nectar, particularly in long-corolla flowers with unexposed nectaries at the flower base, such as hummingbird- or bumblebee-specialized flowers. Nectar microbes are species-poor and taxonomically distinct from other floral microbes, making it relatively straightforward to differentiate target taxa from contaminants.

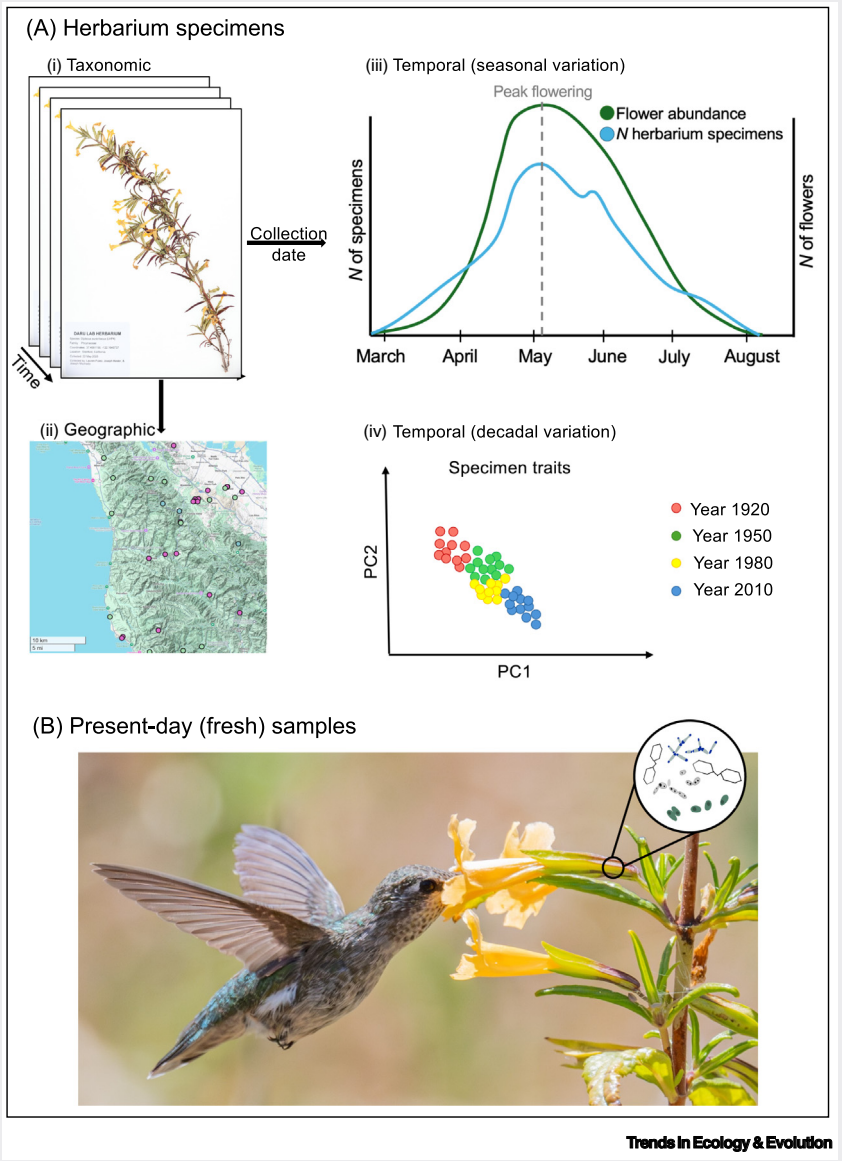

Figure I. (A) Specimens can reveal (i) taxonomic, (ii) geographic, (iii) seasonal, and (iv) decadal variations in species interactions. (B) Present-day collections of plants and floral nectar at matching locations can enable comparisons with historical samples. Image provided by Rick Morris.

dominance, where some species become more influential than others. Taxonomic mismatches potentially create positive feedback loops of cascading changes in pollination-related species interactions.

In some cases, there is a phylogenetic component that structures geographic range or phenological shifts. In other cases, phenotypic plasticity can enable rapid and less-predictable patterns of adaptive response to environmental change. Differential responses to environmental change complicate predictions of pollination systems under anthropogenic pressures. This requires broader taxonomic representation, which is achievable with herbarium collections (Box 1).

The way forward

Herbarium specimens can enable a more fine-resolution analysis of plant–pollinator–microbe interactions. From here, we can model how species interactions respond to different selective pressures to better predict future changes and help address outstanding questions, such as how anthropogenic change has reshaped floral nectar microbiomes and chemistry, and whether these shifts have altered pollination outcomes.

Acknowledgments

This project was supported by grants from the US National Science Foundation (awards 2345994 and 2416314), the Alfred P. Sloan Foundation, the Stanford Woods Institute Big Ideas for Oceans, and the National Institute of General Medical Sciences award NIH T32 GM15466301. We thank the Daru lab members for their feedback and Callie Chappell for the figure artwork.

Declaration of interests

The authors declare no competing interests.

[1]Department of Biology, Stanford University, Stanford, CA 94305, USA
[2]Department of Earth System Science, Stanford University, Stanford, CA 94305, USA

*Correspondence:
lpuleo@stanford.edu (L.H. Puleo) and
bdaru@stanford.edu (B.H. Daru).

https://doi.org/10.1016/j.tree.2026.05.012

References

1. Vega, C. *et al.* (2021) The role of plant–pollinator interactions in structuring nectar microbial communities. *J. Ecol.* 109, 3379–3395
2. Chappell, C.R. *et al.* (2022) Wide-ranging consequences of priority effects governed by an overarching factor. *Elife* 11, e79647
3. Steffan, S.A. *et al.* (2024) Microbes, the 'silent third partners' of bee-angiosperm mutualisms. *Trends Ecol. Evol.* 39, 65–77
4. Phukela, B. *et al.* (2020) A snapshot of evolutionary history of floral nectaries across angiosperm lineages. In *Reproductive Ecology of Flowering Plants: Patterns and Processes* (Tandon, R. *et al.*, eds), pp. 105–129, Springer
5. Settele, J. *et al.* (2016) Climate change impacts on pollination. *Nat. Plants* 2, 16092

6. Abrahamczyk, S. *et al.* (2017) Pollinator adaptation and the evolution of floral nectar sugar composition. *J. Evol. Biol.* 30, 112–127
7. Park, D.S. *et al.* (2018) Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 374, 20170394
8. Daru, B.H. *et al.* (2018) A novel proof of concept for capturing the diversity of endophytic fungi preserved in herbarium specimens. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 374, 20170395
9. Mendes Resende, J.V. *et al.* (2020) Chemical profiling of herbarium samples of solanum (Solanaceae) using mass spectrometry. *Phytochem. Lett.* 36, 99–105
10. Łuczak, P. *et al.* (2023) Stability of fructooligosaccharides in convectively dried fruits after initial osmoconcentration. *Food Bioprocess Technol.* 16, 2511–2520
11. Heberling, J.M. and Burke, D.J. (2019) Utilizing herbarium specimens to quantify historical mycorrhizal communities. *Appl. Plant Sci.* 7, e01223
12. Waters, C. *et al.* (2025) Looking to the past to inform the future: what eDNA from herbarium specimens can tell us about plant–animal interactions. *Appl. Plant Sci.* 13, e11633
13. Johnson, A.L. *et al.* (2019) Pollen on stigmas of herbarium specimens: a window into the impacts of a century of environmental disturbance on pollen transfer. *Am. Nat.* 194, 405–413
14. Young, J.-G. *et al.* (2021) Reconstruction of plant–pollinator networks from observational data. *Nat. Commun.* 12, 3911
15. Zoller, L. *et al.* (2023) Plant–pollinator network change across a century in the subarctic. *Nat. Ecol. Evol.* 7, 102–112