Review

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Tansley review

The developmental basis of floral nectary diversity and evolution

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Summary

Nectar is a central bridge between angiosperms and animal mutualists. It is produced by specialized structures termed nectaries, which can be found on different plant organs. Consumption of floral nectar by pollinators and the subsequent transfer of pollen contribute to the reproductive success of both angiosperms and their pollinators. Floral nectaries have evolved many times independently, feature diverse structural organizations, and produce nectars with various compositions, which cater to a wide range of pollinators. While the nectary and its nectar have been documented for two millennia, many aspects of nectary biology are still unknown. Recent advances in genetics, genomics, and comparative analyses across diverse species have accelerated our understanding of floral nectary structures and the genetic circuits behind their formation and evolution. In this review, we summarize the recent breakthroughs in nectary research and provide a macroevolutionary framework of floral nectary evolution, focusing on the genetic mechanisms that drive nectary development and shape nectary diversity.

I. Introduction

Plants produce various types of secretions, including resins, oils, latex, mucilage, and nectar (Fahn, 1988). Nectar is a sugary reward that acts as the primary currency of plant–animal interactions (Nicolson & Thornburg, 2007; Roy *et al.*, 2017). Observations of nectar and its central role in plant–animal mutualisms date back to Sprengel and Darwin. Generally, nectar secreted by floral nectaries attracts and rewards visitors for their pollination service, while nectar secreted by extrafloral nectaries (those forming on leaves and

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stems) rewards mutualists for defending the plants against herbivores. Beyond these mutualisms, nectar is also a food source for nectar robbers, which obtain nectar without aiding in pollination (Irwin *et al.*, 2010; Richman *et al.*, 2017; Heiling *et al.*, 2018) and serves as an environmental host to diverse microbial communities (Vannette, 2020; Quevedo-Caraballo *et al.*, 2025).

Nectar is more than a combination of sugar and water as it contains a lower abundance of amino acids, proteins, and specialized metabolites. These minor components of nectar not only aid in attracting visitors but also assist in forming specialized interactions between plants and their mutualists (Barberis *et al.*, 2023).

Specific mixtures of specialized metabolites may repel some visitors but attract others (Kessler & Baldwin, 2007), enhance a pollinator's memory for the nectar reward (Wright *et al.*, 2013), and increase nectar consumption (Parkinson *et al.*, 2025). Some nectars contain defensive compounds, such as hydrogen peroxide and terpenoids, which limit microbial proliferation and repel nectar robbers (Adler, 2000; Carter & Thornburg, 2004; Stevenson, 2020; Vannette, 2020; Nicolson, 2022; Mueller *et al.*, 2023). Nectar replenishment and reabsorption may also play a role in preventing microbial growth and maintaining nectar quality (Nepi & Stpiczyńska, 2008).

As the structure that produces nectar, the nectary is at the interface of complex evolutionary and ecological processes, contributing to the overall fitness of all partners (Ackerman *et al.*, 1994; Brandenburg *et al.*, 2012, but also see Irwin *et al.*, 2010; Heiling *et al.*, 2018; Wang *et al.*, 2023; Pyke & Ren, 2023), and represents one of the factors driving plant–animal co-diversification (Lunau, 2004; Crepet & Niklas, 2009; Johnson & Anderson, 2010; Pyke, 2016; Parachnowitsch *et al.*, 2019; McWhorter *et al.*, 2021; Brito Vera & Pérez, 2024; Liu *et al.*, 2024). Numerous studies have examined the structure of nectaries and the cellular mechanisms of nectar secretion throughout angiosperms (Daumann, 1970; Erbar, 2014; Roguz *et al.*, 2018; Phukela *et al.*, 2020; Tölke *et al.*, 2020). In parallel with these structural studies, nectary transcriptomes across phylogenetically diverse species have offered insights into nectary function, and genetic studies in model plant species have isolated genes critical for nectary development and function (Lee *et al.*, 2005; Kram *et al.*, 2009; Liu *et al.*, 2019; Solhaug *et al.*, 2019b; Pei *et al.*, 2021). These studies have provided essential pieces to the puzzle of nectary formation and its evolutionary origins and diversification.

Here, we review the recent advances in nectary research through the lens of developmental genetics. We categorize the types of nectaries based on structure and development, highlight core genetic components that regulate nectary formation and nectar secretion, and summarize the trends of nectary evolution in angiosperms across phylogenetic scales. We employ a developmental framework to address open questions in nectary biology and establish key points of comparison across diverse nectaries and across angiosperms (Fig. 1). While floral and extrafloral nectaries both serve essential roles in plant-biotic interactions, our knowledge of nectary development and function is mostly derived from floral nectaries. For the remainder of this review, we will focus our attention on floral nectaries, and we will use the term nectaries to refer to floral nectaries unless stated otherwise.

II. Diversity of nectary structures

Nectaries have evolved multiple times independently in angiosperms, and many lineages display repeated nectary gain and loss events accompanying shifts in their primary pollinators (Bernardello, 2007; Phukela *et al.*, 2020). Unlike many plant organs and structures, nectaries are defined not by their similar position on the plant nor their anatomical structure, but by their shared biological function – the process of nectar secretion (Fig. 2). This functional definition, combined with the numerous evolutionary derivations of nectaries, means that nectaries are extremely diverse and have sometimes been conflated with other tissues that secrete liquids (e.g. hydathodes, stigmas, various glandular trichomes, and colleters) (Thomas, 1991; Vogel, 1997, 1998a,b,c). However, the primary function of all nectaries is to secrete sugary liquid as a reward for animals, and historically, secretory structures that fulfill such a function are termed nectaries. Although nectar rewards and nectaries have evolved in diverse groups of vascular plants, such as the leaf nectaries of ferns (Mehltreter *et al.*, 2021), the diversity of nectaries is most prominent in angiosperm flowers. It is these floral nectaries that we will focus on here. In angiosperms, most nectaries are well integrated with the rest of the floral organs, making it difficult to define the exact boundaries between nectary and nonnectary tissue without careful histological examination and gene expression data for nectary marker genes.

In general, to fulfill the functions of nectaries, two separate but connected processes need to occur: the production of nectar components and their secretion (Nepi & Stpiczyńska, 2008; Roy *et al.*, 2017). Sugars, the primary component of nectar, can be synthesized in place or transported to the nectary cells. The final form of the secreted sugars often requires several transformation steps inside the nectary cells and additional modifications in the apoplast (Ruhlmann *et al.*, 2010; Lin *et al.*, 2014; Chatt *et al.*, 2021). Along with sugars, minor components (such as amino acids and specialized metabolites) also accumulate in the nectary cells before secretion (Nepi & Stpiczyńska, 2008; Roy *et al.*, 2017). Typically, nectar secretion strongly coincides with flower opening (anthesis). During anthesis, the accumulated sugars, together with other soluble nectar components, are secreted from the nectary cells, which can occur via eccrine (through membrane transporters), granulocrine (using exocytosis), or holocrine (via membrane rupture) pathways (Lin *et al.*, 2014; Antoń & Kamińska, 2015; Chatt *et al.*, 2021; Fig. 2d). While the molecular mechanisms of nectar sugar production appear largely conserved across most angiosperm nectaries, the cellular machineries underlying nectar secretion are more variable and nectary-type dependent.

Structural studies implicate several types of cells in nectar production and secretion: modified epidermal cells that create pathways to the external environment and may be directly involved in nectar production; parenchyma cells that metabolize the organic and inorganic components found in nectar; and vascular tissues that supply the bulk of sugar and water (Fahn, 1979; Erbar, 2014).

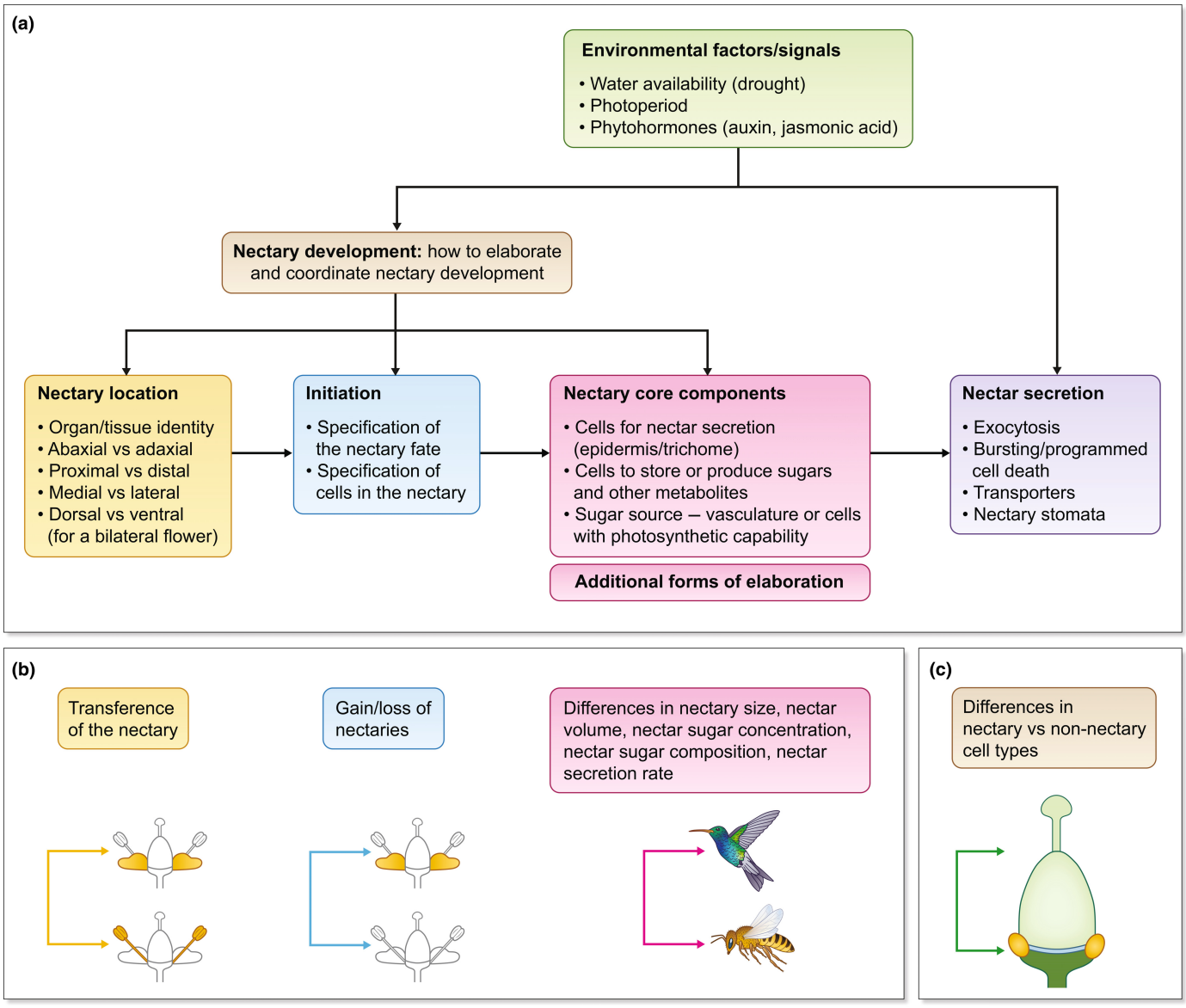

Fig. 1 Evolutionary developmental framework for examining the diversity of floral nectaries and how they form. (a) Map outlining the decisions and inputs required for proper nectary development, from where to position the nectary to the core components of the nectary, the order of nectary development, how nectar exits the nectary, and how external signals are integrated in these processes. (b) Leveraging the diversity of floral nectaries to ask questions about each stage of nectary development: yellow, inputs for determining nectary position can be examined by comparing closely related species with nectaries originating from different organs; blue, inputs for nectary initiation can be examined by comparing species with and without nectaries; pink, differences in the core components of the nectary (size, shape) or nectar (chemical composition, secretion amounts) can be examined by comparing species with different primary pollinators. (c) Comparisons across different nectary (yellow oval) and non-nectary (green) cell types within an organ reveal genetic, molecular, and cellular mechanisms underlying nectary development.

During nectary development, the progenitors of these cells undergo specialized differentiation processes that distinguish them from other cells in the same organ. The spatiotemporal coordination of these differentiation events allows plants to precisely control the timing and extent of nectar secretion.

Depending on the specification and organization of nectary cells, nectaries can be classified into three major types: mesophyllary, trichomatic, and epithelial (Fig. 2c). Mesophyllary nectaries are the most common and the best-studied type because they are present in the model species *Arabidopsis thaliana* (Brassicaceae, Brassicales), *Petunia hybrida* (Solanaceae, Solanales), and tobacco (*Nicotiana tabacum*, Solanaceae, Solanales) (Ge *et al.*, 2000; Lee *et al.*, 2005; Ren *et al.*, 2007a,b). In mesophyllary nectaries, parenchyma cells in the L2 layer produce and secrete nectar into the extracellular space, and stomata in the L1 layer serve as the gateways to the outside environment. Nectary stomata (nectarostomata) are modified in comparison with stomata on the leaf surface as they lack an elaborate gas chamber underneath their opening and do not close

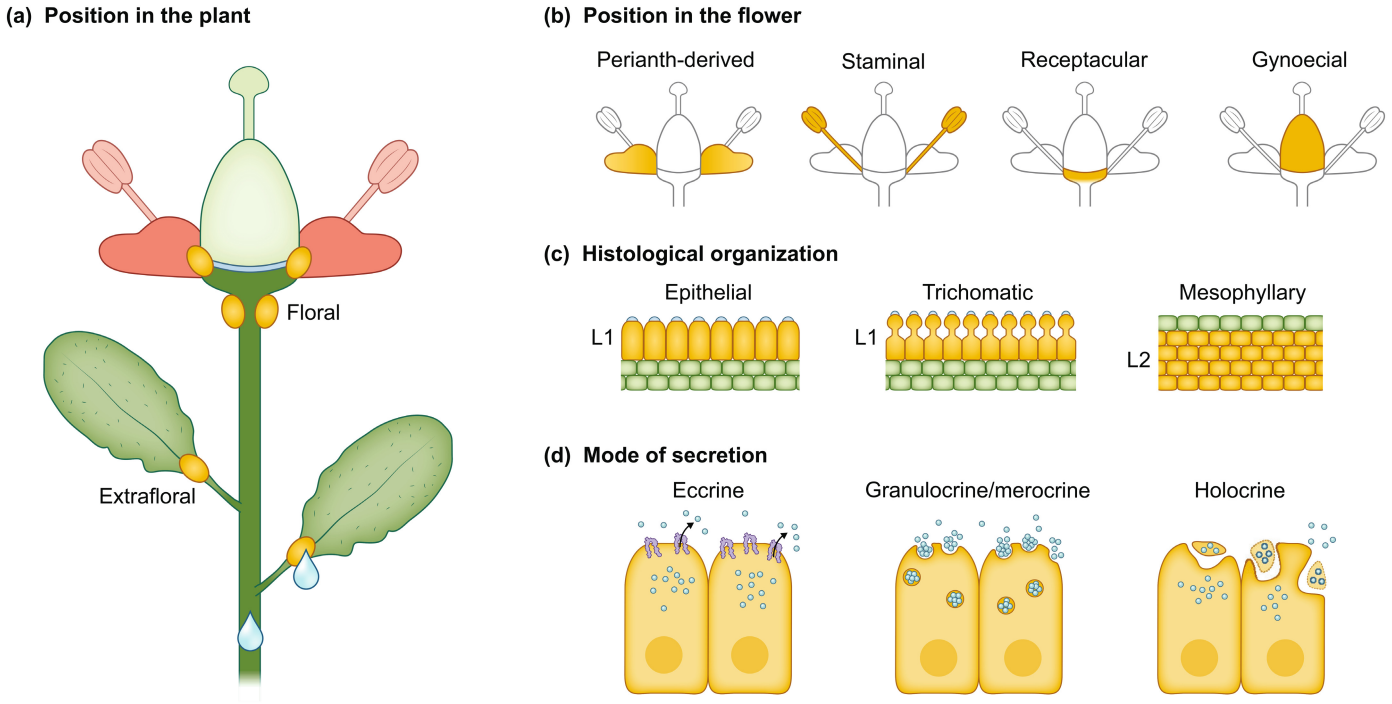

Fig. 2 Nectary diversity across multiple levels of organization. (a) One of the earliest and most commonly used nectary classifications specifies the general location of the nectary (yellow ovals), with floral nectaries present in flowers and extrafloral nectaries present outside of the flower, for example on leaves. The blue droplet without a yellow oval represents a 'structureless' nectary that is capable of secreting nectar. (b) Nectaries (colored in yellow) can be derived from different floral organs, including the perianth (tepal, sepal, or petal), stamens, carpels, and the receptacle. (c) Histologically, the secretory cells of epithelial and trichomatic nectaries are L1-derived (outermost layer of cells), while mesophyllary nectaries are L2-derived (bottom layers of cells). Nectary cells are colored in yellow. (d) Finally, the ultrastructure of the nectary cells reveals the mechanisms of nectar secretion: eccrine glands, which function through the activity of transporters on the cell membrane; granulocrine/merocrine glands that employ vesicular transport; and holocrine glands, which produce nectar through gland autolysis. Blue dots represent nectar and its components.

once open. Trichomatic nectaries produce and secrete nectar through glandular trichomes in the epidermis, which are often multicellular (i.e. *Gossypium* and *Triumfetta* (Malvaceae, Malvales); Espolador Leitão *et al.*, 2005, Chatt *et al.*, 2021). Nectar is secreted by the distal cells of these glandular trichomes into the extracellular spaces beneath the cuticle (often through exocytosis), which causes the cuticle to rupture and release the nectar. This mechanism of secretion is documented in Anacardiaceae (Sapindales), Caprifoliaceae (Dipsacales), and Lentibulariaceae (Lamiales) (Fahn, 1979; Wunnachit *et al.*, 1992; Lustofin *et al.*, 2020). Similar to trichomatic nectaries, epithelial nectaries also secrete nectar through epidermal cells. However, these epidermal cells do not differentiate into trichomes; instead, the nectary epidermal cells rupture to create passages and release the nectar. Notable examples of such nectaries are those from the basal eudicot family Ranunculaceae (Ranunculales), which includes *Aquilegia*, *Aconitum*, and *Helleborus*, among others (Vesprini *et al.*, 1999; Antoń & Kamińska, 2015).

III. Evolutionary aspects of nectary development

Floral nectaries exhibit diverse structural organizations and are associated with different floral organs throughout angiosperms, suggesting that they evolved independently multiple times with many instances of transitions among nectary states and secondary loss of nectaries (Fig. 3). Even when associated with the same floral organ, the structure and precise location of the nectary often vary between lineages, suggesting a dynamic evolutionary history and a lack of positional constraint. For instance, floral nectaries from the monkeyflower (*Erythranthe/Mimulus lewisii*, Phrymaceae, Lamiales) and banana (*Musa acuminata*, Musaceae, Zingiberales) both develop on carpels, but *Mimulus* nectaries form at the base of carpels while *Musa* nectaries form at the inner carpel margins of the gynoecium. Mapping the presence and position of floral nectaries across the angiosperm phylogeny showcases general trends of nectary diversity and serves as an important framework for evolutionary and comparative analyses (Bernardello, 2007; Erbar, 2014; Phukela *et al.*, 2020). Additionally, some lineages exhibit both floral and extrafloral nectaries (e.g. *Gossypium hirsutum*, Malvaceae, Malvales; *Dioscorea alata*, Dioscoreaceae, Dioscoreales), which facilitate different types of plant–animal interactions.

Several studies have mapped the presence and absence of floral nectaries in major plant families to highlight the diversity and general trends of the locations where nectaries are found in a phylogenetic context (Bernardello, 2007; Erbar, 2014; Phukela *et al.*, 2020). With this broad phylogenetic framework, we can begin to address fundamental questions about nectary

development and evolution. What are the origins of nectaries? What determines from where and which organs a nectary develops? How is the nectary function transferred between different organs? How have different angiosperm clades gained or lost nectaries? Some lineages develop both floral and extrafloral nectaries—how are these nectaries structurally, physiologically, and molecularly

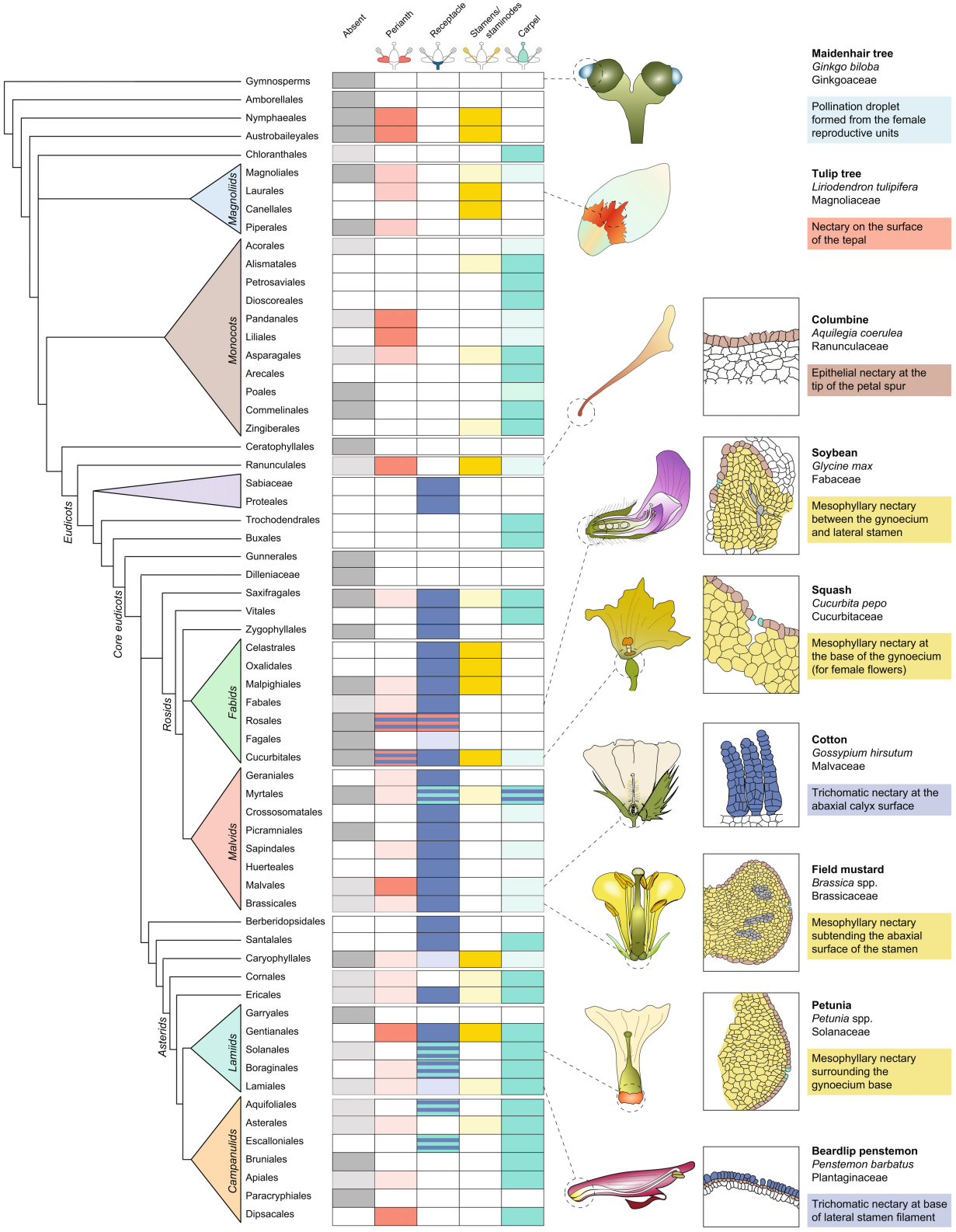

Fig. 3 Diversity of floral nectary position mapped on a phylogenetic tree of seed plants with examples of the histological organization from select species. Character mapping on the APG IV phylogeny (Chase *et al*., 2016) interpreted from Bernardello (2007), Erbar (2014), and Phukela *et al*. (2020). Colored boxes indicate the more common state of the nectary position while lighter shaded boxes indicate a rare occurrence. Nectaries derived from or associated with a shared structure (e.g. hypanthial nectaries in Rosales derived from the perianth or the receptacle) are marked by two colors. Gymnosperms lack nectaries but produce secretions as pollination droplets, which can also serve as animal rewards. Examples of model species for nectary research are on the right with the location of the nectary (circle outline) and images of the associated nectary histology. Colors in the histological images highlight key cell types for each type of nectary: blue, trichomes of a trichomatic nectary and nectary stomata from a mesophyllary nectary; light brown, epidermis of an epidermal nectary or mesophyllary nectary; yellow, parenchyma cells in mesophyllary nectary; gray, vasculature in nectaries.

similar? In what ways are they different enough to maintain specific mutualistic interactions? With a comparative phylogenetic framework and the advances in genetics and genomic technologies, we can begin to make advances in uncovering the origin and diversification of the floral nectary.

1. The origin of the floral nectary

Secretory glands in plant reproductive tissues exist outside of angiosperms, most notably pollination drops in gymnosperms, which are sugary secretions from the micropyle that assist with pollen reception and hydration and that guide the sperm to the ovule (Coulter *et al*., 2012; von Aderkas *et al*., 2018; Prior *et al*., 2019). Some pollination drops have a nectar-like chemical composition, for example containing sugars and amino acids, and may function as pollinator rewards (Nepi *et al*., 2009). In angiosperms, members of the ANITA grade, including *Amborella trichopoda* (Amborellaceae, Amborellales), have stigmatic secretions (Endress & Igersheim, 2000; Thien *et al*., 2003). Although stigmatic secretions and pollination drops have different developmental origins, both have similar functions in assisting pre-fertilization processes between the pollen and the ovule. It is plausible that the common ancestor of angiosperms produced floral secretions as a secondary reward in the form of stigmatic exudates from the open surface of the carpel, with pollen as the major reward for pollinators, such as beetles (Thien *et al*., 2003; Erbar, 2014). When examining other members of the ANITA grade, floral nectaries in extant lineages such as *Cabomba* (Cambobaceae, Nymphaeales), *Nuphar* (Nymphaeaceae, Nymphaeales), and *Illicium* (Schisandraceae, Austrobaileyales) (Thien *et al*., 2009), are generally found on the perianth, although some taxa possess both perianth-derived nectaries and produce stigmatic secretions (Endress, 2008; Thien *et al*., 2009; Erbar, 2014) (Fig. 3).

Several hypotheses have been proposed for the origin of nectar (reviewed in De la Barrera & Nobel, 2004). According to the 'leaky phloem' hypothesis, phloem solution leaks through structural weaknesses in cell walls during tissue growth. The 'sugar secretion' hypothesis suggests that excess solutes in the phloem build up in the nectary and are excreted as nectar. Support for the 'leaky phloem' hypothesis is found in Andean *Melastomataceae* species (Myrtales). These species do not have specialized nectary structures or glands; rather, phloem sap derived from a central vascular bundle is released at the abaxial surfaces of stamen filaments (Vogel, 1997). While this is likely a more recent developmental innovation, it offers an intriguing case study to consider for the origins of floral nectaries.

2. Phylogenetic trends in floral nectary location

The location of nectaries exhibits several general trends among the four major angiosperm clades –magnoliids, monocots, rosids, and asterids (Bernardello, 2007; Erbar, 2014; Phukela *et al*., 2020) (Fig. 2). At a broad scale, magnoliids typically have perianth or stamen-associated nectaries, and the majority of the monocot orders have gynoecium-derived septal nectaries found at the flanks of the fused carpels (Smets *et al*., 2000; Remizowa *et al*., 2010; Tobe *et al*., 2018). While nectary position in the core eudicots appears to be associated with any floral organ, in *Arabidopsis* and many rosids, nectaries typically form on the receptacle at the base of the floral organs, with substantial variation in their position relative to the stamen whorl. These nectaries tend to be mesophyllary, with notable exceptions such as cotton, which develops trichomatic nectaries on the sepal. In *Petunia*, *Mimulus*, and many asterids, nectaries are associated with the gynoecium. These may develop on the outer surface of the carpel, or derive from the receptacle and form a 'disc' around the ovary (e.g. *Ipomoea*, Convolvulaceae, Solanales).

Some lineages exhibit remarkable conservation of nectary location and structure, whereas in other clades, nectary position and form are variable. As mentioned above, most nectar-secreting monocots form nectaries internally between the margins of fused carpels, which are termed septal or gynopleural nectaries (Fig. 4) (Smets *et al*., 2000; Remizowa *et al*., 2010; Tobe *et al*., 2018). In most monocots, carpels initiate individually and become partially fused postgenitally (van Heel, 1988; Vogel, 1998; Rudall, 2002); thus, the regions where the carpels remain unfused are where most monocot nectaries develop. Most septal nectaries are composed of a secretory epidermal layer that lacks stomata or trichomes. Nectar is secreted into the lumen and exits through ducts, slits, or secretory pores at the plant surface. Character mapping suggests that the septal nectary has a single origin in monocots (Tobe *et al*., 2018) (Fig. 3), suggesting that there are developmental and genetic constraints on nectary structure and position in monocots despite wide variation in primary pollinators and environmental factors.

By contrast, many eudicot lineages have much more dynamic nectary evolution. For example, the basal eudicot order Ranunculales harbors a large diversity of variously positioned nectaries and exhibits frequent nectary gain and loss (Carrive *et al*., 2020). Of the seven families of this order, both Ranunculaceae and Berberidaceae form nectaries primarily on their elaborated petals, which often undergo extensive three-dimensional growth into various shapes to hold nectar.

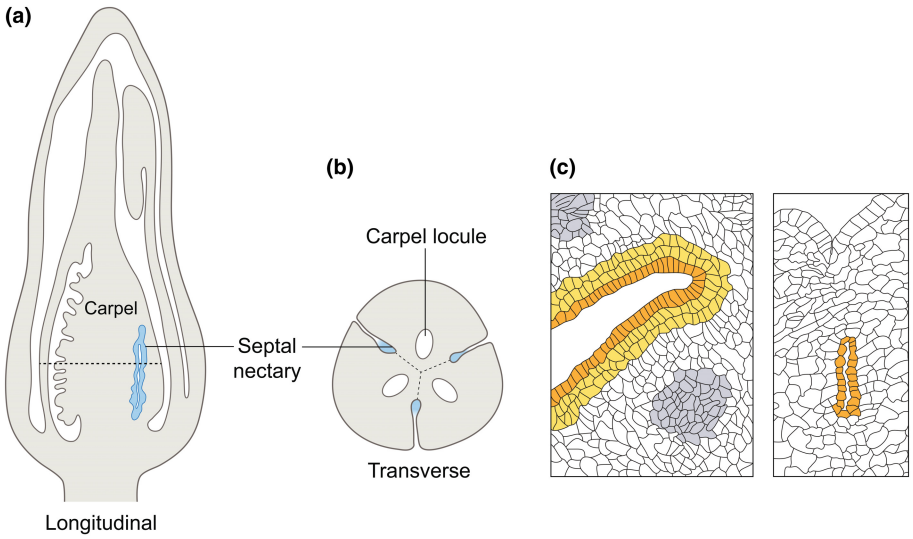

Fig. 4 Monocots typically have septal nectaries. (a) Longitudinal cross-section of a monocot flower with the septal nectary highlighted in blue. (b) Transverse cross-section through the gynoecium, with the septal nectary found in the unfused margins between the three carpels. Blue represents the nectar secreted into the space created by the unfused carpel margins. (c) Two anatomical tracings from a transverse section of the septal nectary from *Aechmea gamosepala* (Bromeliaceae; Poales, left) and *Asparagus officinalis* (Asparagaceae; Asparagales, right) showing cellular features: peach represents the epidermal layers of the nectary, yellow the subepidermal parenchymal layers, and gray the carpel vascular bundles.

No secretory trichomes or stomata are found on the epidermis of these nectaries, with holocrine secretion suggested based on histological studies (Antoń & Kamińska, 2015). In Ranunculaceae, due to the subfunctionalization of the B-class gene *AP3-3* that controls petal identity, petals may be lost without pleiotropic effects. Thus, petals undergo dynamic patterns of gain and loss, which also result in a gain and loss of nectaries and frequent shifts in nectary positions (Zhang *et al.*, 2013). For instance, *Caltha palustris* (Ranunculaceae) lacks petals, yet trichomatic nectaries form on the carpel (Peterson *et al.*, 1979). Similar to Ranunculaceae, many Papaveraceae develop nectar-holding spurs in the perianth organs, and the nectar is secreted from nectaries at the base of the stamens (Erbar, 2014; Zhang & Zhao, 2018). No specialized epidermal structures nor programmed cell death is observed on these nectaries, and their nectar secretion mechanism has remained a mystery. Together, the rich structural diversity of Ranunculales nectaries and the mysterious nectary secretion mode of some of their members make them a fascinating system for future investigation of the evolution and the developmental mechanisms of nectaries.

Of course, many angiosperm lineages lack nectaries, either ancestrally or due to secondary loss. For instance, species-poor sister lineages of major angiosperm clades generally lack nectaries. In Acorales, sister to the rest of the monocots, pollen is the primary reward for pollinators (Funamoto *et al.*, 2020), while in Ceratophyllales, sister to the eudicots, they are aquatic and exhibit a water pollination strategy (Gottsberger, 2015). It is generally believed that secondary losses of nectaries are due to the inherent cost of nectar production (Pyke & Ren, 2023). These events are associated with transitions from animal-dependent pollination to wind, water, and self-pollination or are associated with changes in primary pollinator reward from nectar to pollen or oils (Smets *et al.*, 2000). For instance, nectary loss can be inferred in most wind-pollinated lineages, such as Fagales (e.g. oaks) and Poales (e.g. grasses), and in groups with specialized modes of pollination (buzz pollination in *Solanum* (Vallejo-Marín, 2019), shifts to other secretory rewards such as oils in Cucurbitaceae (Cucurbitales) and scent in Orchidaceae (Asparagales)) (Renner & Schaefer, 2010; Tölke *et al.*, 2020).

IV. Genetic mechanisms of nectary development

Because of the independent origins of floral nectaries in different clades, their frequent evolutionary gains and losses, and the positional and structural diversity of nectaries, the genetic basis of nectary development is likely not conserved across all angiosperms. However, work in model core eudicot species has suggested similar regulators of nectary development, such as the transcription factor (TF) *CRABS CLAW* (*CRC*) (Bowman & Smyth, 1999), and similar functional components in the secretory process, including the sucrose transporter *SWEET9* (Lin *et al.*, 2014), and *CELL WALL INVERTASE 2/4* (*CWIN2/4*) (Ruhlmann *et al.*, 2010; Minami *et al.*, 2021) (Fig. 5). This surprising degree of similarity could be attributed to the fact that these nectaries are all found in comparable positions on the floral organs and that similar metabolic pathways are employed to produce and secrete sugary nectar, propounding a case for genetic convergence. For instance, nectaries that form on the abaxial surface of floral organs likely express abaxial polarity factors. Likewise, trichomatic nectaries likely employ similar trichome differentiation pathways regardless of their organ association. Intriguingly, the recurrent use of these genes could also suggest a potential modularity of nectary development and function, where the activation of an evolutionarily conserved 'core nectary regulatory widget' at specific positions of the plant body is sufficient to drive the formation of functional nectaries. To further examine whether these patterns evolved convergently or resulted from the deployment of an ancestral nectary module, characterization of the nectary regulatory networks across the angiosperms is needed. Comparing these networks may explain the frequent, independent evolution of nectaries in angiosperms and the recruitment of similar genetic factors that pattern these structures.

Previous investigations of the molecular and genetic mechanisms controlling nectary development have focused on two major

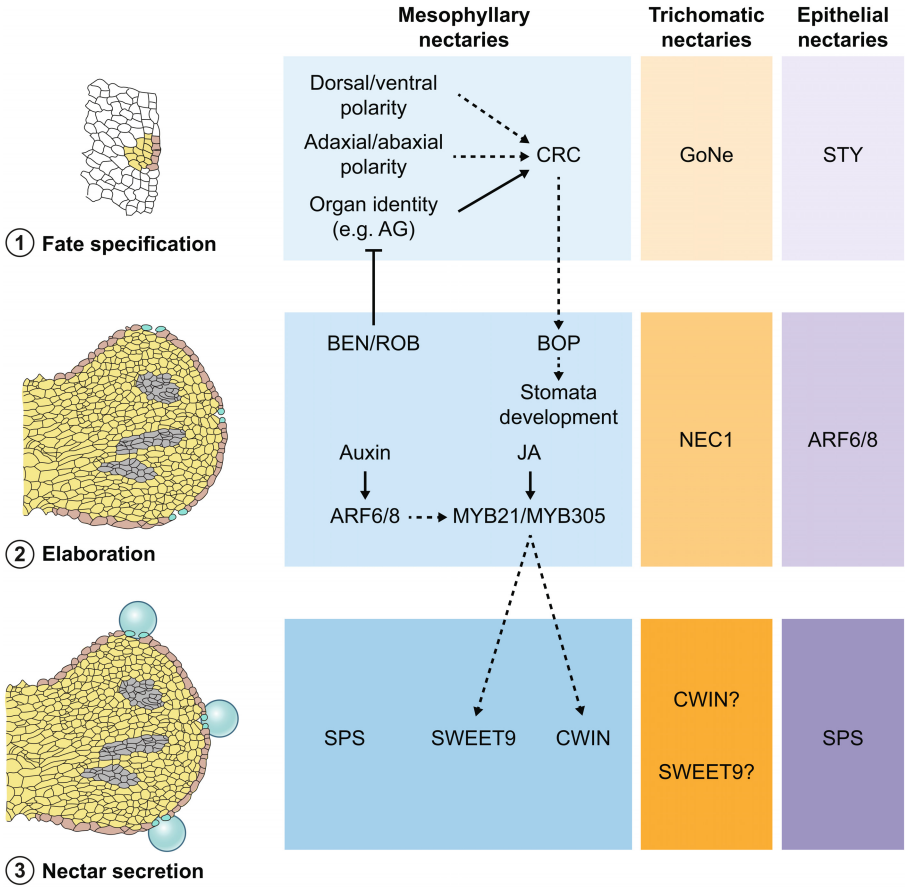

Fig. 5 Nectary developmental stages and key regulators for different types of nectaries. Many genes have been identified as regulators of nectary development, which can be divided into three stages: fate specification, elaboration, and nectar secretion. Direct regulation is labeled with solid lines while indirect and unvalidated interactions are labeled in dashed lines. Information from mesophyllary nectaries synthesizes research in *Arabidopsis*, *Nicotiana*, *Petunia*, and *Mimulus* (schematics on the left); information for trichomatic nectaries is based on *Gossypium* and for epithelial nectaries on research in *Aquilegia* (see text for further citations).

developmental aspects: the specification of nectary fate and the elaboration of nectary structure. Transcriptional profiling and forward mutagenesis screens have yielded core components of these processes. The core genetic modules and molecular pathways that mediate nectary development and nectar secretion (Fig. 5) are mostly derived from research in the mesophyllary nectaries of *Arabidopsis* and *Petunia*, which may not be (and are probably not) representative of trichomatic and epithelial nectaries.

1. Specification of the nectary fate

Nectary initiation in several species appears to be under the control of the TF *CRC* (Bowman & Smyth, 1999). *CRC* is a member of the plant-specific TF YABBY family, which is involved in establishing the polarity of lateral organs. Unlike most YABBY TFs, *CRC* orthologs across the angiosperms typically lack vegetative expression and are primarily expressed in carpels, where they retain the gene family's ancestral role in specifying abaxial cell fate. *CRC* is also expressed in the nectaries of many core eudicot species, including the floral nectaries of *Cleome* (Cleomaceae, Brassicales) and *Nicotiana* as well as the extrafloral nectaries of *Capparis* (Capparaceae, Brassicales) and *Gossypium* (Malvaceae, Malvales) (Lee *et al.*, 2005). Loss-of-function mutants of *CRC* in the rosid *Arabidopsis* and the asterid *Petunia*, both species with mesophyllary nectaries, fail to develop nectaries (Bowman & Smyth, 1999; Morel *et al.*, 2018). These observations have prompted the hypothesis that *CRC*'s role in nectary development is conserved across core eudicots, despite the likely independent evolution of nectaries in rosids and asterids. Notably, many species with reported *CRC* expression in the nectary develop nectaries on the abaxial side of their associated organ – stamens in *Arabidopsis* and carpels in *Petunia* (Lee *et al.*, 2005; Morel *et al.*, 2018) – suggesting an alternative hypothesis: the recruitment of *CRC* in nectaries reflects abaxial placement rather than functional conservation. It is important to note that ectopic *CRC* expression is not sufficient for nectary formation in or outside of the flower (Baum *et al.*, 2001) and additional, currently unknown, factors are required. Determining whether these additional factors are shared among species with independently derived nectaries will shed light on the evolutionary conservation of the 'core nectary regulatory widget'.

CRABS CLAW has dual functions in carpel development and nectary initiation and differentiation, and distinct upstream regulators and *cis*-regulatory elements are likely responsible for specifying its nectary expression. Phylogenetic footprinting analysis comparing the *CRC* promoter in three Brassicaceae species identified discrete conserved regions that are required for nectary or carpel activation of *CRC* (Lee *et al.*, 2005). MADS-box TF binding sites (CarG boxes) were found in the conserved promoter regions of the three species, and mutating some of these binding sites abolished *CRC* promoter activity in the nectary. Further studies in both *Arabidopsis* and *Petunia* demonstrated that eliminating the primary C-class function gene *AGAMOUS* (*AG*)

and its paralogs *SHATTERPROOF1/2* disrupted nectary formation (Morel *et al.*, 2018). Chromatin immunoprecipitation in *Arabidopsis* also showed that C-class (AG) and B-class (APETALA3 (AP3), PISTILLATA (PI)) MADS-box TFs bind *in vivo* to the *CRC* promoter (Ó'Maoiléidigh *et al.*, 2013). Together, these observations highlight the significance of MADS-box genes in *CRC* activation and nectary development and provide a mechanistic connection between floral organ identity and nectary development.

Studies in core eudicot lineages outside of Brassicaceae and Solanaceae have revealed other regulators required for nectary development. In *Gossypium*, quantitative trait locus (QTL) mapping of cotton varieties with and without nectaries identified *GoNe*, an APETALA 2/ethylene-responsive factor (AP2/ERF) required for the formation of both floral and extrafloral trichomatic nectaries (Pei *et al.*, 2021). While *CRC* was reported to be expressed in cotton nectaries (Lee *et al.*, 2005) and it is expressed in the extrafloral nectaries of *GoNe* mutants (Pei *et al.*, 2021), no *crc* mutant or knockdown lines have been studied, and it is unknown whether *CRC* is required for specifying nectary fate in cotton. It is also unclear whether the *GoNe* function is specific to trichomatic nectary development.

Less is known about genetic regulators of nectary initiation and development outside of the core eudicots. Based on expression, it appears that the *CRC* homolog in *Aquilegia* (Ranunculaceae, basal eudicot) retains a role in carpel development, but it is not expressed in the epithelial nectary that forms at the tips of petal spurs (Lee *et al.*, 2005; Min *et al.*, 2019). Instead, nectary development in Ranunculaceae, and likely its sister family Berberidaceae, requires members of an unrelated TF family, *SHORT INTERNODES/STYLISH* (*STY*). Interestingly, *STY* has a conserved role in the morphogenesis of the apical part of the carpel in angiosperms (Min *et al.*, 2019). The recruitment of conserved factors involved in organ morphogenesis, such as *CRC* and *STY*, in nectary development suggests that motifs of the nectary regulatory network have been co-opted from pre-existing organ morphogenesis networks to engage downstream factors responsible for nectary placement or nectar secretion.

There is limited expression and no functional data on nectary development in angiosperm clades outside of the eudicots. Transcriptional profiling of the perianth-derived nectaries in a magnoliid, *Liriodendron* (Magnoliaceae), did not detect expression of *CRC* homologs (Liu *et al.*, 2019). Likewise, in the monocot *Asparagus* (Asparagaceae, Asparagales), a *CRC* homolog, *DROOPING LEAF*, retains its ancestral expression in the abaxial surface of the carpel but is not expressed in septal nectaries (Nakayama *et al.*, 2010). Broader genomic examination of nectary development in noneudicots may reveal novel regulators of nectary fate initiation.

2. Elaboration of the nectary structure

After initiation, the formation of a functional nectary requires structural elaboration to form the major cell types necessary for producing and secreting nectar, which manifests as coordinated cell growth and differentiation. These cellular processes contribute to the overall morphology of the nectary as well as its size and shape. Genetic studies have identified several genes and gene families that play a role in nectary elaboration. Additionally, quantitative genetics is yielding clues about the degree of genetic and evolutionary complexity necessary for the differentiation of these structures between closely related species. The transcriptional coactivators *BLADE-ON-PETIOLE1/2* (*BOP1/2*), which were first identified as abscission zone/tissue polarity regulators in *Arabidopsis* (Ha *et al.*, 2003), contribute to proper nectary development. In the *Arabidopsis bop1bop2* double mutant, nectaries are initiated but never fully mature: secretory parenchyma cells do not differentiate, nectary stomata do not form, and no nectar is secreted (McKim *et al.*, 2008). Given that *CRC* is still expressed in these nonfunctional nectaries, it is likely that *BOP1/2* act downstream of *CRC* in the nectary developmental program. Similarly, in peas (*Pisum sativum*, Fabaceae, Fabales), mutation in a *BOP* homolog also causes defects in nectary development (Sinjushin, 2022), suggesting that *BOP1/2* function in either initiation or elaboration of the nectary in pea flowers and may serve a conserved role in nectary development in rosids.

The plant hormones auxin and jasmonate play significant roles in regulating nectary development and nectar secretion. The involvement of auxin in nectar production has been recognized since the 1950s (Roy *et al.*, 2017). This is further supported by the observation that the auxin signaling reporter DR5::GUS is highly active in *Arabidopsis* nectaries (Aloni *et al.*, 2006). Furthermore, several auxin transporter and signaling genes (*PIN6*, *ARF6*, and *ARF8*) exhibit enriched expression in the nectaries of diverse eudicot taxa, including *Arabidopsis*, *Cleome*, and *Aquilegia*; mutants of these auxin-related genes often develop nonfunctional and reduced nectaries (Reeves *et al.*, 2012; Bender *et al.*, 2013; Zhang *et al.*, 2020; Carey *et al.*, 2023). Exogenous application of auxin and jasmonic acid in some taxa can induce nectar production (Radhika *et al.*, 2010), and the mutants of several genes involved in jasmonic acid signaling (*Arabidopsis MYB21*/*Nicotiana MYB305*, *AOS*, *DAD1*, *COI*, and *NEC1*) exhibit defects in nectary maturation and nectar secretion (Ge *et al.*, 2000; Liu *et al.*, 2009; Liu & Thornburg, 2012; Schmitt *et al.*, 2018; Hu *et al.*, 2020). Furthermore, ARF6 and ARF8 are relevant to both pathways and integrate auxin and jasmonic acid signaling (Reeves *et al.*, 2012). It remains to be determined how hormone signaling integration takes place in the nectary developmental network.

Nectary size, which is constrained by floral size and floral organ arrangement, requires the coordination of cell division and proliferation. In *Petunia*, two AP2-like genes, *BLIND ENHANCER* (*BEN*) and *REPRESSOR OF B-FUNCTION* (*ROB*), function as negative regulators of nectary size, which appears to be determined by C-class gene dosage (Morel *et al.*, 2018). QTL studies in two *Ipomoea* species with different mating systems and different nectary sizes have provided evidence for a complex genetic architecture of nectary size differences (Liao *et al.*, 2022). In *Ipomoea*, nectary size is moderately genetically correlated with flower size, with a few shared overlapping QTLs, suggesting that nectary size is partly dependent on the size of the overall flower or the organ on which it develops.

Changes in nectary size are correlated with the amount of nectar and nectar sugar produced and are typically driven by differences in

primary pollinators or mating systems. For instance, in *Penstemon* (Plantaginaceae, Lamiales) species, larger nectaries are associated with the hummingbird-pollinated *P. kunthii* compared to the bee-adapted *P. amphorellae* (Katzer *et al.*, 2019); nectary size is also positively associated with the nectar volume produced, in line with hummingbird preferences for copious amounts of dilute nectar. QTL studies reveal similar patterns in *Aquilegia* species (Edwards *et al.*, 2021). In highly selfing *Ipomoea* (Galetto & Bernardello, 2004) and *Nicotiana* (Kaczorowski *et al.*, 2005) species, nectary size, nectar volume, and nectar sugar concentration are all reduced. These genetic correlations suggest that pleiotropy or tight gene linkage is responsible for this suite of nectar traits, which may facilitate their evolution (Wessinger & Hileman, 2016) and ultimately aid in the identification of genetic regulators underlying nectary size differences.

V. Mechanisms of nectar secretion and their structural considerations

At the core of nectary development is the coordination of molecular and cellular functions for producing and secreting nectar. Nectar is primarily composed of water and simple sugars – sucrose, glucose, and fructose – and its hexose-to-sucrose ratio varies due to pollinator preferences (McWhorter *et al.*, 2021; Nicolson, 2022; Liu *et al.*, 2024). Regardless of nectary type, nectar production processes often include starch buildup, production of simple sugars, and sugar transport (Fig. 6).

Nearly all nectary types accumulate starch in the amyloplasts of developing nectaries, which is the major source of nectar sugar; the starch granules break down rapidly before anthesis (Horner *et al.*, 2003, 2007; Ren *et al.*, 2007a; Antoń & Kamińska, 2015; Chatt *et al.*, 2021). Starch in the nectary has two major sources: sugar translocated from the phloem or synthesized locally via photosynthesis. The relative contribution of phloem-derived sugars and locally synthesized sugars to the accumulation of starch in the nectaries likely varies depending on the plant species, environmental conditions, and developmental stage. Of these two sources, phloem sugar is more common, whereas local sugar and starch synthesis through photosynthesis is highly debated, despite many nectaries being green (Lüttge, 2013; Clearwater *et al.*, 2021). Intermediate sugar storage in starch granules in the developing nectaries has been attributed to the general organization of vasculature in the nectaries: vascular bundles rarely extend into the nectariferous tissue to ensure a continuous sucrose concentration gradient. Thus, using nectary-localized starch as an intermediate provides tighter spatiotemporal regulation of nectar production and coordination with other morphological and physiological changes during anthesis.

Early microarray and RNA-seq profiling of gene expression in *Arabidopsis*, *Nicotiana*, *Cucurbita*, and *Gossypium* nectaries have suggested the involvement of similar starch biosynthesis and metabolic genes (e.g. sucrose synthase, starch synthase, starch branching enzymes, starch debranching enzymes, and amylase) in these species (Ren *et al.*, 2007a; Kram *et al.*, 2009; Solhaug *et al.*, 2019b; Chatt *et al.*, 2021). The expression of homologous genes in distantly related plant species with likely independently derived nectaries suggests conservation in the metabolic pathways underlying nectar production across diverse plant lineages. The expression of genes involved in starch metabolism is often tightly regulated and occurs in waves (Ren *et al.*, 2007b; Solhaug *et al.*, 2019a), which is likely coordinated by the circadian clock and photosynthesis source–sink dynamics, although the molecular basis of this temporal regulation has not been fully characterized.

The total sugar concentration of nectar can be very high, reaching up to 80% in some cases (Roy *et al.*, 2017), but typically ranges between 10% and 60%. Such high sugar concentrations are impossible to achieve through simple diffusion. In the best-studied mesophyllary nectaries, starch degradation in the L2 layer parenchyma cells precedes sucrose synthesis by SUCROSE-PHOSPHATE SYNTHASE (SPS) (Lin *et al.*, 2014). Sucrose is then transported out of nectary cells into the apoplastic space via facilitated diffusion by *SWEET9*, which is a sucrose-specific uniporter that requires a concentration gradient for sugar transport (Lin *et al.*, 2014; Chen *et al.*, 2015; Tao *et al.*, 2015). Thus, to maintain sucrose transport by *SWEET9*, it is necessary to maintain a high sucrose concentration inside the cells and a low sucrose concentration outside. To these ends, SPS synthesizes sucrose intercellularly and *CWIN2/4* cleaves extracellular sucrose

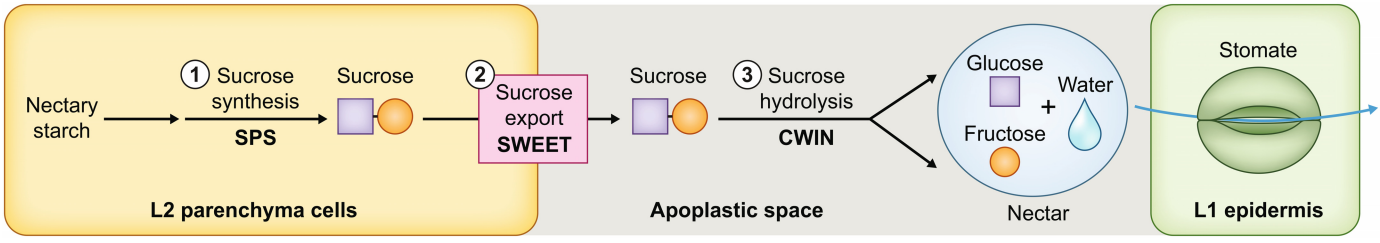

Fig. 6 SPS-SWEET9-CWIN framework of nectar secretion in mesophyllary nectaries. Among all nectary types, the nectar secretion of mesophyllary nectaries is the best understood. Nectary starch builds up in the parenchyma cells of the L2 layers during nectary development. Before anthesis, starch is hydrolyzed via the activities of alpha-amylase, beta-amylase, starch-debranching enzymes, and other hydrolases. Sucrose is re-synthesized by sucrose phosphate synthase (SPS) using UDP-glucose and fructose, transported into the apoplastic space by sugar transporters (e.g. SWEET9), and hydrolyzed by cell wall invertase (CWIN) to glucose and fructose. Glucose, fructose, and residual sucrose generate osmotic pressure in the apoplast, and water is drawn out from the surrounding cells to mix with the sugar and form nectar. Nectar eventually leaks out of the nectary stomata to the plant surface, where it is accessible to pollinators.

into glucose and fructose, thereby exhausting the sucrose extracellular pool. The increase in the concentration of hexoses (glucose and fructose) increases osmotic pressure in the apoplast, allowing water to be drawn out from the surrounding cells and nectar is formed (Kram *et al.*, 2009; Ruhlmann *et al.*, 2010). Nectar eventually leaks out of the nectariferous tissue through nectary stomata on the epidermis. Loss-of-function mutations in *SPS1/2*, *SWEET9*, or *CWIN2/4* in *Arabidopsis* result in a lack of nectar production, validating that sucrose export and turnover are essential for nectar production (Ruhlmann *et al.*, 2010; Lin *et al.*, 2014). Additionally, the expression levels and activity of *CWIN* are correlated with nectar sugar concentration and the ratio of sucrose to hexoses in different species of *Nicotiana* (Tiedge & Lohaus, 2018). Reducing *CWIN4a* expression level in *Brassica* also significantly reduces nectar volume and alters nectar sugar composition (Minami *et al.*, 2021). These results suggest that each node of the SPS-SWEET-CWIN pathway can act as a rheostat to control the output of nectar secretion in nature when plants experience shifts in primary pollinator preferences and in agriculture to engineer crops with different amounts of nectar.

In the trichomatic nectaries of *Gossypium*, starch accumulates in the parenchyma cells beneath the secretory trichomes, and sugar molecules from starch degradation are transported into the secretory trichomes through extensive plasmodesmatal connections (Chatt *et al.*, 2021). It is hypothesized that nectar metabolites are then packaged into vesicles and secreted through exocytosis. The secreted nectar accumulates between the cell wall and the cuticle, and exits the nectary through the rupture of the cuticle (Fig. 1e; Nepi, 2007). This model is based solely on histological observation. Interestingly, transcriptional profiling has shown that the same starch and sugar metabolism genes (e.g. *CWIN* and *SWEET9*) are active in *Gossypium* nectaries (Chatt *et al.*, 2021), suggesting that there is a generalized nectar production module associated with sugar processing and secretion that has been repetitively activated in independently evolved nectaries.

Besides ultrastructural evidence, little is known about the molecular mechanism behind nectar secretion for the epithelial type. Phylogenetic analysis revealed that the *SWEET9* clade is specifically absent in noncore eudicot lineages such as *Aquilegia* (Lin *et al.*, 2014), opening the possibility that other *SWEET* family homologs could have been co-opted for sugar transport. It would be interesting to examine whether other known components of nectar sugar production are expressed in *Aquilegia* nectaries. Given that *Aquilegia* nectar is secreted through cell bursting, it is possible that nectar production of sugars has similar genetic factors, but unrelated factors are involved in generating the osmotic pressure for cell bursting and nectar release to the outer surface.

Comparative studies between closely related species with different nectar attributes offer additional genetic insights into nectar production and secretion processes. For instance, hummingbird-adapted flowers produce a more dilute nectar, whereas that from bee-adapted flowers is more concentrated (Faegri & Van Der Pijl, 1979; Wilson *et al.*, 2004). QTL studies have shown that these differences in nectar volume and nectar sugar concentration between closely related species in *Aquilegia* and *Penstemon* have a relatively simple genetic basis, with a few major loci contributing to a large proportion of the variance (Wessinger *et al.*, 2014; Edwards *et al.*, 2021). Conversely, shifts to self-pollination result in a complex genetic basis for nectar volume and nectar sugar concentration in closely related *Ipomoea* species (Liao *et al.*, 2022). The genes involved in nectar volume and nectar sugar concentration appear to be coordinated – many QTL studies show a genetic correlation between these two traits regardless of whether the correlation is positive or negative (Wessinger *et al.*, 2014; Kostyun *et al.*, 2019; Edwards *et al.*, 2021; Rifkin *et al.*, 2021; Liao *et al.*, 2022). Although the underlying gene(s) have not been identified, these types of comparative studies in diverse species offer clues to understanding the overall processes underlying nectar production, secretion, and development.

In some lineages, nectar secretion dynamics also include the processes of nectar replenishment and reabsorption. Both nectar replenishment after removal and reabsorption after fertilization are considered to alleviate the high energy cost of nectar production and could contribute to limiting microbial growth (Stpiczyńska & Nepi, 2006; Nepi & Stpiczyńska, 2008). While the mechanism of nectar secretion is well studied, we have limited knowledge of how replenishment and/or reabsorption are controlled at the cellular and molecular levels.

Nectar is more than just sugars and water – it often includes amino acids, proteins, fatty acids, lipids, specialized metabolites, and salts (Nicolson & Thornburg, 2007; Roy *et al.*, 2017; Nicolson, 2022). Due to its nutrient-rich environment, nectar also harbors a complex microbiome. These commonly neglected parts of nectar can play significant roles in maintaining nectar homeostasis, deterring nectar robbers, facilitating pollinator attraction, and limiting microbe over-proliferation (Adler, 2000; Stevenson, 2020; Vannette, 2020; Nicolson, 2022; Mueller *et al.*, 2023). A few plant lineages have also evolved colored nectar to attract a large variety of pollinators, including insects, birds, lizards, and mammals (Hansen *et al.*, 2007). The diversity of nectar chemistry, including the presence of colored nectar and the effects of nectar microbes in altering the metabolic composition, is beyond the scope of this review; insights can be found in Roy *et al.* (2017), Nicolson (2022), and Magner *et al.* (2025, doi: 10.1111/nph.70031).

VI. Nectary positioning

Among all the open questions regarding nectary development and function, perhaps one of the most intriguing ones is the mechanism behind nectary location determination. Nectaries often develop after the identity of other floral organs has been established and organ differentiation has been initiated (Smets *et al.*, 2000). For instance, in *A. thaliana*, the floral nectaries develop at floral stage 9, after carpel fusion and stamen locule formation (Smyth *et al.*, 1990). In other species, floral nectary development similarly occurs after the initiation of other floral organs (Ge *et al.*, 2000; Ren *et al.*, 2007a). As a result, the nectary can be associated with and develop from any part of the flower. These observations are consistent with the existence of a core genetic module controlling nectary development that is recruited to different floral organs, depending on factors such as floral architecture and types of pollinators that these species attract.

CRABS CLAW is a key factor expressed in nectaries localized in different parts of the flower: it is expressed in floral nectaries found in the first whorl (*Gossypium*, on the abaxial surface of the sepal), the third whorl (*Arabidopsis*, at the base and outside of the stamens), and the fourth whorl (*Nicotiana*, on the abaxial surface of the carpel). The nectary location is likely determined by the upstream regulators of *CRC* in the gene regulatory network controlling nectary initiation. In *Arabidopsis*, C-class genes (*AG*, *SHP1/2*), along with E-class (*SEPALLATA*) genes, likely act upstream of *CRC* to mediate nectary development (Baum *et al.*, 2001; Lee *et al.*, 2005). C-class activity is also required for stamen identity, consistent with the association between stamens and the nectary in *Arabidopsis*. Interestingly, in *Arabidopsis* floral homeotic mutants, nectaries still develop at the base of the third whorl (Baum *et al.*, 2001), which suggests the existence of factors instructing the positional placement of the nectary rather than the association with a particular organ identity.

As a YABBY TF, *CRC* regulates organ abaxial-adaxial polarity specifically in the carpel (Bowman & Smyth, 1999; Siegfried *et al.*, 1999). In both *Arabidopsis* and *Petunia*, where nectaries are associated with different floral organs, late *CRC* expression and nectary formation are in the abaxial position (Bowman & Smyth, 1999; Lee *et al.*, 2005; Morel *et al.*, 2018). Furthermore, in monosymmetric flowers (e.g. *Mimulus*, *Cleome*, and *Tropaeolum* (Tropaeolaceae, Brassicales)), where nectaries form on one side of the dorsal-ventral axis (Rachmilevitz & Fahn, 1975; Carey *et al.*, 2023), the genetic pathway underlying floral zygomorphy (*CYCLOIDEA-RADIALIS* module) likely acts upstream of the core *CRC* nectary initiation module to constrain its location.

It is unclear how nectary locations are conferred in lineages that develop nectaries independent of CRC function. No *CRC* expression is found in the nectaries of *Liriodendron* (Liu *et al.*, 2019) or *Aquilegia* (Min *et al.*, 2019), both of which develop in the perianth. Future studies on the upstream regulatory networks of nectary fate in these taxa are crucial to understanding the diverse nectary location determination mechanisms in angiosperms.

Given the role of nectar as a reward for pollinators, nectaries are frequently positioned to encourage deep floral exploration. Thus, despite the diversity in floral organ association or its histological organization, nectaries are often found at the base of floral organs, requiring pollinators to delve into the flower to reach the nectar source. Specialized structures associated with the nectaries store and allow nectar to accumulate. Examples of these modifications range from petal and sepal spurs (*Aquilegia* and *Tropaeolum*), curved tepals in unfused perianths (*Musa*), fused corolla tubes (*Mimulus* and *Nicotiana*), saccate sepals in Brassicaceae (Nikolov, 2019), and patches of trichomes or tissue extensions that form a pouch (*Gossypium* and *Gloriosa* (Colchicaceae, Lilales)). In several cases, the nectary is not immediately within or adjacent to the site of nectar accumulation. For instance, in toadflax (*Linaria*, Plantaginaceae, Lamiales), the nectary is found at the base of the pistil, but nectar accumulates in the perianth spur delivered by nectar ducts (Vogel, 1998). Additional floral elaborations, such as nectar guides, may direct pollinators to the nectar source (Free, 1970; Leonard *et al.*, 2013). The distance between the nectar accumulation site and the flower opening dictates the extent to which pollinators have to reach into the flower to retrieve the reward and indicates coevolution between the plant and the primary pollinator. Classic examples include Darwin's orchid (*Angraecum sesquipedale*, Orchidaceae, Asparagales) and sphinx moth (*Xanthopan morganii praedicta*) (Arditti *et al.*, 2012) and the positive correlation between the length of the *Aquilegia* petal spurs and their primary pollinator species (Whittall & Hodges, 2007).

VII. Conclusions and future directions

The production and secretion of floral nectar is one of several key innovations in angiosperms (Crepet & Niklas, 2009; McWhorter *et al.*, 2021), allowing lineages to exploit their environment in a novel way by mediating pollinator interactions and facilitating sexual reproduction (Miller *et al.*, 2023). As the specialized organs that produce nectar, nectaries continue to attract the attention of biologists. Modern molecular genetics and imaging approaches have begun to uncover the developmental principles, cellular processes, and molecular pathways behind nectary formation and nectar secretion across angiosperms. However, these recent advances are taxonomically isolated and primarily derived from a small number of model species. A comprehensive characterization across phylogenetically diverse lineages is needed to obtain a systematic understanding of nectary biology. With the establishment of additional genetic models for studying nectaries, traditional forward genetics approaches will help identify other essential components underlying nectary development.

In model species, several transcriptional factors (CRC, STY, and GoNe) have been identified as the essential regulators of nectary development. However, none of these factors are yet known to be sufficient to promote nectary identity. In the case of *CRC*, its activity alone is not sufficient to initiate nectaries, while this has yet to be tested with *GoNe* in cotton and *STY* in *Aquilegia*. It will be interesting to determine whether a 'master regulator' of nectary identity exists or whether nectary identity is dependent on synergistic interactions with other factors, including organ identity pathways. Additionally, the extent to which nectary regulators function in a nectary-type-specific manner remains unclear. Do these TFs share similar downstream targets? Does CRC function similarly in mesophyllary nectaries and trichomatic nectaries? Transcription factors integrate a variety of signals and are well positioned to provide insight into the upstream events that determine the location and timing of nectary development as well as the downstream molecular pathways responsible for specifying the type and size of the nectary. High-resolution techniques that can capture rare and difficult-to-study cell types and states, such as single-cell transcriptional and chromatin accessibility profiling, may reveal novel factors and provide insight into the regulatory networks controlling nectary development in diverse nectary types and plant lineages.

From the onset of nectary initiation to the completion of nectar secretion, nectaries undergo a series of coordinated cell proliferation and differentiation processes across multiple cell layers and organ developmental stages. Classic histological and anatomical characterization continues to be a valuable resource for describing the cellular heterogeneity of nectaries (Erbar, 2024; Erbar &

Söte, 2024). Other advanced imaging techniques, such as micro-computed tomography (microCT) and confocal live imaging approaches, further shed light on the differentiation dynamics of the nectary cell types and the specific nectary morphologies. Such imaging modalities will provide platforms for the functional characterization of mutants, the natural variation of nectary structure and development, the coordination between the development of nectaries and other floral organs, and the overall integration of floral functions, such as organ vascularization.

The SPS-SWEET-CWIN framework outlines the key enzymes and transporters for nectar secretion. However, it is unclear whether this functional framework operates only in mesophyllary nectaries or is shared by all nectary types. How the nectar production pathway varies in trichomatic and epithelial nectaries remains to be determined. While the transport of sugars in nectar is relatively well studied, the mechanisms by which other nectar metabolites, such as amino acids and specialized metabolites, are incorporated in the nectar remain largely unknown. Determining whether these transport processes are coordinated with sugar and water transport is crucial for understanding nectar secretion.

Nectar replenishment and reabsorption may be some of the most fascinating but unstudied aspects of nectar biology. While nectar replenishment and reabsorption have been documented in various taxa, we do not know the cellular or molecular basis of these processes. How does the nectary sense when to replenish or reabsorb nectar? Is the circadian clock involved? How do pollination signals influence these processes? Are there specialized cells and dedicated transporters for nectar uptake? Are the same nectar production pathways involved? What is the ecological significance of replenishing or reabsorbing nectar?

From an evolutionary perspective, the independent and frequent origin of floral nectaries in diverse taxa presents both challenges and unique opportunities to understand the origin of floral nectaries and their dynamic evolution. How similar at the genetic level are independently derived nectaries in different lineages associated with the same floral organs? What are the constraints that lead to structural similarities in nectary organization across lineages? Within the same organism, how are floral and extrafloral nectaries structurally, physiologically, and molecularly similar and different?

Throughout this review, we have outlined a framework to study nectary formation within a single species and to uncover the balance between conservation and divergence in nectary development and function through a comparative approach across closely and distantly related species. The fundamental stages for nectary development – defining the nectary domain within the flower, initiating nectary fate, coordinating nectary morphogenesis, and fine-tuning the cellular processes involved in the production and secretion of nectar – are shared among species, but the underlying genetic mechanisms controlling these processes are expected to differ in independently derived nectaries. We need to move beyond classifying nectaries based on their location, cellular type, and secretion process, and rather take a holistic view of nectary development and functions, incorporating both molecular evidence and evolutionary trends. Ultimately, by combining traditional and new genetic, genomic, and cell biological approaches within a developmental and evolutionary framework, advances can be made toward understanding the mechanisms contributing to the evolution of these important structures.

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Competing interests

None declared.

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