

Arabidopsis thaliana as a model for functional nectary analysis

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Abstract Nectaries and nectar have received much research attention for well over 200 years due to their central roles in plant–pollinator interactions. Despite this, only a few genes have demonstrated impacts on nectary development, and none have been reported to mediate de novo nectar production. This scarcity of information is largely due to the lack of a model that combines sizeable nectaries, and high levels of nectar production, along with suitable genomics resources. For example, even though *Arabidopsis thaliana* has been useful for developmental studies, it has been largely overlooked as a model for studying nectary function due to the small size of its flowers. However, *Arabidopsis* nectaries, along with those of related species, are quite operational and can be used to discern molecular mechanisms of nectary form and function. A current understanding of the machinery underlying nectary function in plants is briefly presented, with emphasis placed on the prospects of using *Arabidopsis* as a model for studying these processes.

Keywords Nectar · Nectaries · Nectary · *Arabidopsis thaliana*

Introduction

Nectar is a widely offered floral reward secreted by plant organs, termed nectaries, to attract pollinators (Jolivet 1992). Significantly, there is a well-known correlation between nectar quality (e.g., volume and composition), pollinator visitation, and overall pollination efficiency in multiple plant–pollinator systems (e.g., Baker and Baker 1973, 1982; Galliot et al. 2006a; Stuurman et al. 2004). A relationship also appears to exist between floral and nectary form and nectar quality (Davis et al. 1996, 1998; Davis 2001; Nepi 2007; Pacini and Nepi 2007). For example, in a study examining the relationship between floral structures and nectar-carbohydrate production, Davis (2001) identified several characteristics that seem to be associated with high nectar-carbohydrate production. A thickened peduncle with extensive vascularization, a receptacle with large dimensions, and increased nectary size are among these characteristics. With further study, plant lines exhibiting enhanced nectar-carbohydrate production could be selectively bred on the basis of the aforementioned structural features.

Remarkably, the molecular events involved in the synthesis and secretion of nectar, and even the development of floral nectaries, are relatively poorly understood. Indeed, to date, no genes have been reported to directly affect the de novo production of floral nectar. Furthermore, only three genes—*CRABS CLAW* (*CRC*), *BLADE-ON-PETIOLE* (*BOP*) 1, and *BOP*2—have confirmed involvement in the development of normal nectaries (Bowman and Smyth 1999; McKim et al. 2008). With this dearth of information in hand, it is clear that many of the genes participating in de novo nectar production are unknown. To address this gap in knowledge, several research groups (including our own) have initiated projects to elucidate the underlying

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mechanisms of nectary development and function in multiple systems (described in the following paragraphs). Determining the molecular basis of nectar synthesis and secretion can have broad implications, ranging from understanding the co-evolution of plant–pollinator interactions to increasing yields in multiple pollinator-dependent crop species.

Our current understanding of nectary function at a molecular level is relatively limited due to the fact that no model plant, with a completely sequenced genome, offers a substantial amount of nectariferous tissue or produces an appreciable volume of nectar for empirical analysis. For example, ornamental tobacco (Carter et al. 1999; Ren et al. 2007a) and petunia flowers (Stuurman et al. 2004), both of which are extremely useful models for nectary studies, have large nectaries that can produce in excess of 30 μL of nectar per flower, which allows one to easily collect samples, yet these systems have relatively few genomics resources when compared to model plants with completely sequenced genomes. On the other hand, *Arabidopsis thaliana* is an outstanding model plant in terms of genetic and genomic resources, but it produces extremely small volumes of nectar from its minute nectaries. While *Arabidopsis* flowers are indeed diminutive, its nectaries are quite functional and, despite the challenges associated with collecting sufficient nectar or nectary samples, these organs can be functionally studied at the molecular level. This article aims to summarize past reports and current attempts to elucidate molecular mechanisms underlying nectary development and function in multiple plant species, with an emphasis placed on *Arabidopsis* as a useful model system for these purposes. Ecological and evolutionary considerations pertaining to nectaries and nectar are not discussed in depth here, but have been excellently reviewed recently (Brandenburg et al. 2009).

Nectar composition

Floral nectar is mostly comprised of sugars, between 8 and 80% (w/w) depending on the species (Baker and Baker 1983), and also usually contains an array of additional components, including: alkaloids (Deinzer et al. 1977), amino acids (Baker and Baker 1973), flavonoids (Ferrerres et al. 1996), free fatty acids (Kram et al. 2008), glycosides (Roshchina and Roshchina 1993), metal ions (Heinrich 1989), oils (Vogel 1969), organic acids (Baker and Baker 1975), phenolics (Ferrerres et al. 1996), proteins (Carter and Thornburg 2004), terpenes (Ecroyd et al. 1995), and vitamins (Griebel and Hess 1990). The functions of these non-sugar compounds vary considerably, ranging from rendering nectar unpalatable to nectar thieves (Baker 1978) to warding off microbial infection (Carter and Thornburg

2004; Pichersky and Gershenzon 2002), as well as providing additional nutrition to pollinators (Rathman et al. 1990; Rusterholz and Erhardt 2000; Weber 1958).

Sucrose, glucose, and fructose are the most prevalent carbohydrates found in nectar (Baker and Baker 1983). Various nectars also contain other simple carbohydrates in minor concentrations, which are thought to play a role in providing supplementary nutrition to pollinators (Baker and Baker 1983). Davis et al. (1998) previously demonstrated that *Arabidopsis* nectar is hexose-dominant, with the Columbia ecotype displaying a hexose (glucose + fructose)-to-sucrose ratio close to 33.33. This is in direct contrast to the carbohydrate found in *Arabidopsis* phloem sap, which is almost exclusively comprised of sucrose (e.g., Deeken et al. 2002). The compositional differences between nectar and phloem photosynthate in multiple species imply that the phloem ‘pre-nectar’ is modified to yield ‘mature’ nectar, and indeed this proposed process has been supported by a number of studies (Davis et al. 1998; Ren et al. 2007a; Wenzler et al. 2008). Interestingly, the carbohydrate ratio of exuded nectar can differ according to nectary type, within the same flower (i.e., lateral vs. median nectaries; see following description). For the Columbia ecotype, the glucose/fructose ratio of nectar secreted by lateral nectaries averaged 1.15, but was only 0.9 for nectar secreted by median nectaries (Davis et al. 1998). This finding, a difference in carbohydrate ratio between lateral and median nectaries, was consistent across several Brassicaceae species examined (Davis et al. 1998). The mechanics controlling nectar composition and secretion are currently under investigation by a number of groups, with some prevailing notions described herein.

Nectary structure

As indicated earlier in this article, the floral organ responsible for nectar synthesis and secretion is the nectary. Nectaries can occur in different areas of flowers and can take on diverse forms in different species, even to the point of being useful for taxonomic classification (Fahn 1979a). Four general types of nectaries have been reported to occur in Brassicaceae flowers, including: (1) annular: a continuous ring of tissue fused to the ovary at the base of the flower; (2) two-nectary type, with two lateral nectaries at the floral base; (3) four-nectary type, with one pair each of both lateral and median nectaries; and, (4) eight nectary type, with two pairs each of both median and lateral nectaries (Bernardello 2007; Davis et al. 1996, 1998). *Arabidopsis thaliana* flowers generally contain four nectaries, which, as alluded to earlier, consist of two non-equivalent sets of organs (Fig. 1; Davis et al. 1998). One of these sets is known as lateral nectaries and the other

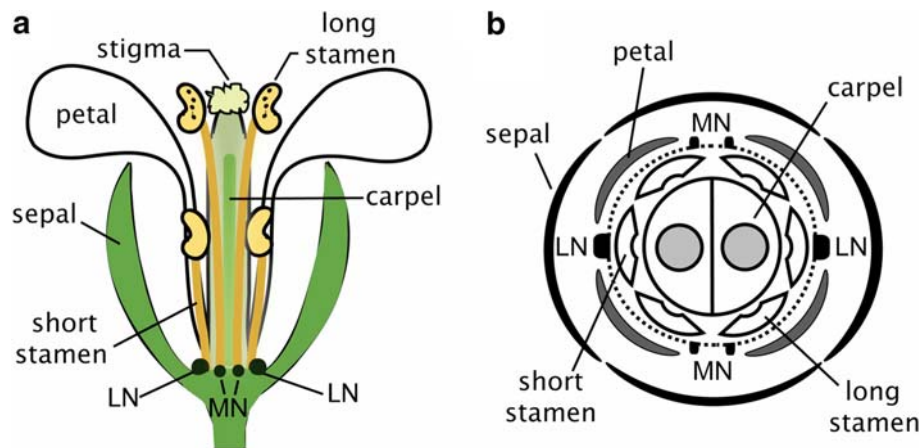


Fig. 1 Schematic of *Arabidopsis thaliana* nectarium. *Arabidopsis* flowers have four nectaries that comprise the ‘nectarium’; two lateral nectaries (LN) occur at the base of short stamens, and two bilobed median nectaries (MN) occur in between the insertion points of two long stamens. **a** Schematic of *Arabidopsis* flower with front sepal and petals not shown (modified from Kram et al. 2009). **b** Schematic

cross-section of flower, from (a), with relative location of floral organs indicated (modified from Davis et al. 1998). A narrow ridge of tissue that occasionally connects median and lateral nectaries is indicated with *dashed lines*. Lateral nectaries produce >95% of total nectar in most Brassicaceae flowers, with median nectaries often being, at best, weakly secretory

as median nectaries (occasionally referred to as ‘medial nectaries’). In *Arabidopsis*, the two lateral nectaries, which are often bilobed, are longitudinally opposed to one another just outside the base of each short stamen and are bounded by petal insertion sites. Currently, there exists some debate as to whether a single flower has two or four median nectaries; regardless, the median nectaries almost always appear bilobed, with each lobe occurring in between the insertion points of a long stamen and a petal. Significantly, even among nectaries sharing a common spatial orientation, nectary morphology can vary significantly, and median and lateral nectaries occasionally appear to be linked to one another by a narrow ridge of tissue (Davis AR, in Bowman 1994).

Nectary vasculature also can significantly differ between species; out of 366 species examined, 39.6% lacked any vascularization, 47.8% were supplied exclusively by phloem, and 12.6% contained both xylem and phloem (Fahn 1979a). In *Arabidopsis*, both lateral and median nectaries are well-differentiated organs, and while they are subtended exclusively by phloem, the nectary parenchyma itself generally contains little vasculature; furthermore, only the lateral nectary is supplied by sieve tubes to an appreciable extent (Davis AR, in Bowman 1994). As previously mentioned, median and lateral nectaries are not equivalent—indeed, it is generally thought that only lateral nectaries significantly contribute to the production of nectar in most *Brassica* species (Davis et al. 1998; Nieuwhof 1969). Nectar volume measurements by Davis et al. (1998) reveal that lateral nectaries produce 96–100% of the total nectar carbohydrate secreted by *Arabidopsis* flowers, while the poorly secreting median nectaries produce the

remaining 0–4%. Vascular differences between lateral and median nectaries are likely at least partially responsible for the disparity in nectar production by these organs (Davis et al. 1986, 1996). Further descriptions of nectary ultrastructure in relation to nectar production are described later on.

While not the primary focus of this article, extrafloral nectaries also play a role in providing rewards to insects. However, in this circumstance, the role of the insect is not to aid in pollination, but rather to defend the plant from herbivory (Heil et al. 2001). As the function and anatomy/physiology of floral and extrafloral nectaries are similar, it is tempting to postulate that similar genetic programming controls the development and functioning of these organs. In evolutionary terms, it has even been suggested that floral and extrafloral nectaries are closely linked (De la Barrera and Nobel 2004). Unfortunately, *Arabidopsis* does not possess extrafloral nectaries, so this plant model cannot be used to gain insight on the similarities and differences of floral and extrafloral nectaries. Further similarities between floral and extrafloral nectaries are briefly discussed in the following paragraphs.

Nectary development

Arabidopsis nectaries do not start developing until ~3.5 days before anthesis and undergo a rapid expansion prior to flower opening (Smyth et al. 1990). It is known that the YABBY family transcription factor *CRABS CLAW* (*CRC*) is required for the initial development of nectaries and carpels in *Arabidopsis thaliana*, and probably for many

other dicots as well (Baum et al. 2001; Bowman and Smyth 1999; Lee et al. 2005a, b). These studies indicate that several positive and negative regulators control *CRC* expression; in particular, it has been proposed that B-class (*APETALA3* and *PISTILLATA*) and C-class (*AGAMOUS*) genes along with *SEPALLATA* coordinate the activation of *CRC* in nectaries and carpels (Lee et al. 2005a). Expression of *CRC* persists at high levels in nectaries long after development, and throughout the secretory process; thus, *CRC* could potentially play an indirect role in the regulation of nectar synthesis and secretion. However, since *crc* mutants lack nectaries it is not understood what effect, if any, this gene has on de novo nectar production. Significantly, downstream *CRC* targets and mechanistic signaling mechanisms regulating nectary development and function are largely unknown at the moment. Like *CRC*, *BOP1* and *2* are transcription factors that are required for proper nectary development. It was recently demonstrated that *bop1/2* double mutants fail to form normal nectaries, and instead develop minor projections at the base of the stamens that lack any typical nectary characteristics (McKim et al. 2008). Since *CRC* expression is retained in *bop1/2* double mutants, the lack of nectary outgrowth is not a consequence of *CRC* deregulation, but instead *BOP 1 and 2* might be acting with *CRC* to promote normal nectary development (McKim et al. 2008).

Nectary ultrastructure and nectar secretion

Nectaries undergo remarkable morphological and metabolic changes during the course of floral development. For example, it is known that pre-secretory nectaries in multiple species, including *Arabidopsis*, accumulate large amounts of starch in amyloplasts (e.g., Fahn 1979a, b, 1988; Ge et al. 2000; Ren et al. 2007a). The ultimate supplier of most pre-nectar sugars stored within these plastids appears to be phloem (schematically shown in Fig. 2), though some starch may be produced in situ via photosynthesis (Davis et al. 1986). Just prior to anthesis and nectar secretion, a rapid degradation of the starch granules occurs, which likely provides much of the carbohydrate that is present in mature nectar (Peng et al. 2004; Ren et al. 2007a; Zhu et al. 1997). However, transport of simple sugars via phloem for secretion, without prior storage in amyloplasts, is also likely in some species (e.g., Wenzler et al. 2008). In the latter instance, pre-nectar still appears to be modified by nectaries prior to secretion (Wenzler et al. 2008).

Even with the aforementioned knowledge in mind, the specific mechanisms of how sugars and other metabolites are transported, and ultimately secreted, are still in question (a greatly generalized schematic is presented in Fig. 2). A

prevailing view of merocrine-type secretion (apparently used by the nectaries of most species, including *Arabidopsis*) suggests that pre-nectar metabolites derived from vascular bundles are transported symplastically via plasmodesmata to nectary parenchyma cells (Fig. 2, step 3) where the sugars are temporarily stored as starch as described earlier (step 4; also reviewed in: Fahn 1979a, b; Pacini and Nepi 2007). In *Arabidopsis*, it appears that CELL WALL INVERTASE 4 (*CWINV4*) helps to maintain a constant sink status in pre-anthesis nectaries via extracellular hydrolysis of sucrose (step 1; also see Fig. 3; Ruhlmann et al. submitted). When needed, starch grains are degraded, their products modified by endoplasmic reticulum and Golgi, and packaged into vesicles that fuse with the plasma membrane via granulocrine-type secretion. Indeed, ultrastructural analyses have repeatedly demonstrated the presence of extensive ER and Golgi networks in nectary secretory cells (Fahn 1979a, b, 1988; Ge et al. 2000; Zhu et al. 1997). Significantly, this theory does not necessarily discount involvement of plasma membrane transporters in generating nectar via direct eccrine-type secretion. Indeed, discerning between granulocrine- and eccrine-type secretion has not been easy up to this point, as both mechanisms have been found to occur and can vary between species (Wist and Davis 2006, 2008). Complicating these analyses is the fact that simultaneous apoplastic flow of sugars supplied from sieve tubes, without prior storage in amyloplasts, cannot be discounted (Pacini and Nepi 2007). The specific point of secretion from the nectary in most instances is thought to be modified stomata, which remain permanently open and provide a direct path out of the nectary (e.g., Fig. 4; Davis AR, in Bowman 1994; Zhu et al. 1995; Zhu and Hu 2002). It should also be noted that a highly reticulated cuticle covers the nectary epidermis in *Arabidopsis* (Davis AR, in Bowman 1994; Nepi 2007), as is often the case for other species (Durkee 1983). Finally, it is important to mention that secretion by a minority of nectary types is mediated by rupture of nectar-containing cells through a process known as ‘holocrine’ secretion (reviewed in Durkee 1983). Moreover, nectar has been demonstrated to be resorbed by nectaries in a number of instances (reviewed in Nepi and Stpiczynska 2008).

Interestingly, due to dense staining patterns as observed by transmission electron microscopy, it has been suggested that *Arabidopsis* nectary parenchyma cells undergo degeneration well before secretion (Zhu et al. 1997; Zhu and Hu 2002); this conclusion is intriguing for a number of reasons. In particular, GUS staining patterns (Tholl et al. 2005), RNA isolation and microarray experiments (Kram et al. 2009), starch staining patterns (Ren et al. 2007a), confocal microscopy studies (see data in upcoming paragraphs), and other studies (Fallahi et al. 2008), suggest that

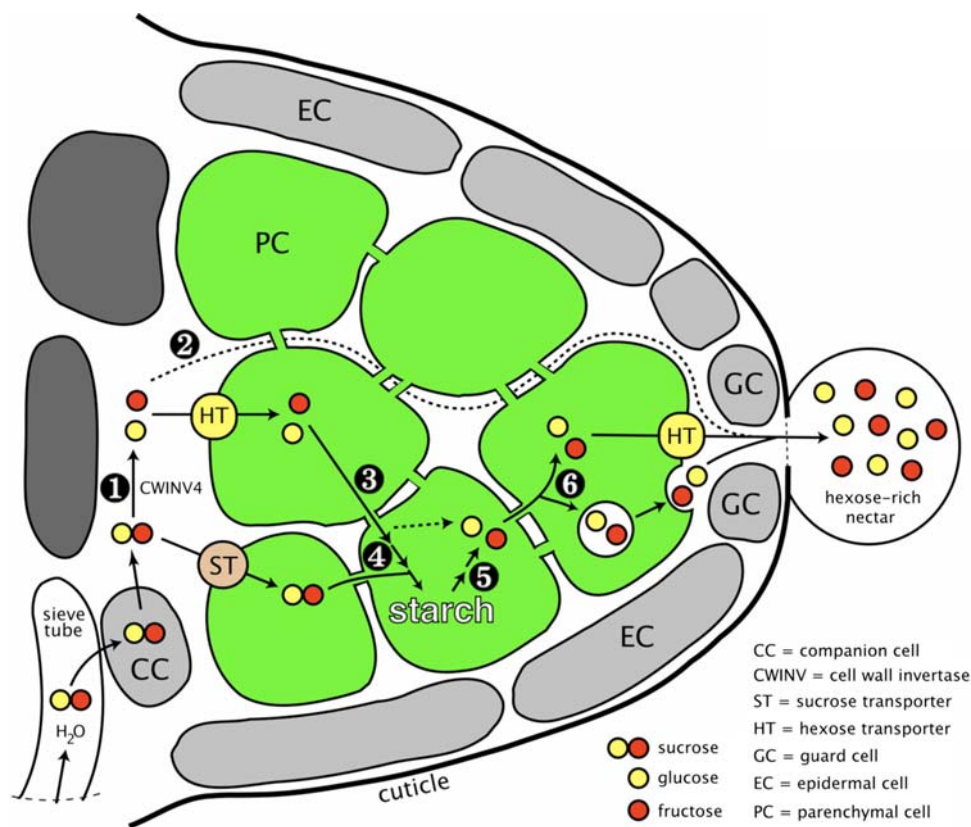


Fig. 2 Nectar synthesis and secretion pathways in *Arabidopsis* and related species. Sieve tubes supply *Arabidopsis* nectaries with pre-nectar (sucrose and other metabolites), which can then take several alternative routes prior to nectar secretion. (1) Phloem-derived sucrose appears to be hydrolyzed into glucose and fructose by CELL WALL INVERTASE 4 (CWINV4) in both pre- and post-anthesis flowers, thus allowing nectaries to maintain a constant sink status (Ruhlmann et al. submitted). The resultant hexoses then either (2) move apoplastically toward stomatal apertures in actively secreting nectaries or (3) are taken up by parenchyma cells and move symplastically via plasmodesmata until (4) they are stored as starch

in pre-anthesis amyloplasts. Shortly before flower opening, (5) starch stores are degraded to yield hexose monomers. The resultant sugars (6) exit the parenchyma cells either via hexose transporters, which translocate sugars into the apoplastic space (eccrine secretion), or are packaged into vesicles that then fuse with the plasma membrane and release the sugars into the apoplast (granulocrine secretion). It is also likely that CWINV4 is involved in the cleavage of sucrose into glucose and fructose in actively secreting cells. The mature hexose-rich nectar ultimately results in water flow out of cells and the resulting mixture is secreted through permanently open stomata

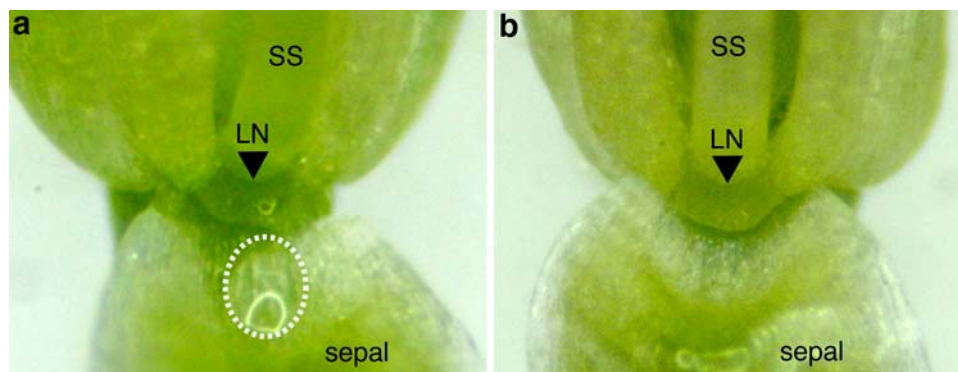


Fig. 3 Evaluation of nectar production in *Arabidopsis* flowers. Gently peeling back the sepal allows for the easy determination of relative increases or decreases in nectar production in *Arabidopsis* flowers. **a** Nectar droplets accumulating within the sepal cups surrounding lateral nectaries (LN) are consistently present in wild-

type plants (circled). **b** *cwinv4* mutants do not secrete nectar (e.g., *cwinv4-1*, SALK_130163). Preliminary analyses indicate that other nectary-specific gene mutants produce little or no nectar, whereas some have increased nectar volumes

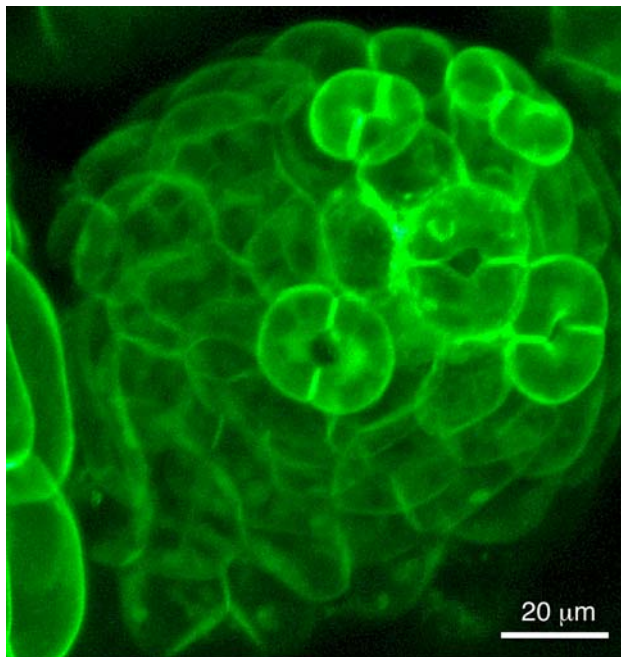


Fig. 4 Analysis of Arabidopsis nectary structure and ultrastructure via laser-scanning confocal microscopy. In vivo imaging of Arabidopsis nectaries is possible with GFP fusion proteins and fluorescent dyes. In this example, laser-scanning confocal microscopy was used to examine an Arabidopsis lateral nectary expressing plasma membrane localized GFP (GFP:LTI6b; described in Cutler et al. 2000). The image shown was compiled from a z-stack of 72 individual photos. Sample preparation consisted simply of removing sepals from the flower prior to imaging. Available software also allows the creation of time-lapse movies to follow membrane and organelle dynamics. Modified stomata are clearly visible and serve as the presumed sites of nectar secretion

the parenchyma cells may not degenerate prior to nectar secretion, or even shortly thereafter. Moreover, Arabidopsis nectaries are sites of auxin synthesis long after nectar secretion has ceased, even up to the point of silique maturation (Aloni et al. 2006).

As stated earlier, significant changes in intracellular morphology, particularly in ER, Golgi, vacuoles, and plastids, are known to occur in nectaries throughout development. To date, and to our knowledge, all reports of nectary ultrastructure have relied on thin sectioning of nectaries followed by light or electron microscopy. However, a significant advantage of Arabidopsis is that nectaries are very small (~100 microns wide and deep) and occur just inside the sepals at the base of the stamen and petals. Thus, simple removal of sepals allows direct access to nectary visualization without further dissection (e.g., Fig. 3). To take advantage of these characteristics, Arabidopsis nectary ultrastructure can be studied in vivo by laser-scanning confocal microscopy (LSCM, Fig. 4). We have examined a large number of transgenic Arabidopsis lines expressing GFP and YFP fusions (Cutler

et al. 2000; Di Sansebastiano et al. 2001; Grebe et al. 2003; Tian et al. 2004; Wang et al. 2004) via LSCM (e.g., Fig. 4). Each of these fusion proteins is targeted to a specific subcellular location, and all have shown good imaging results in nectaries. Plastids are also readily imaged through chlorophyll autofluorescence. In addition to these fluorescent transgenic lines, imaging can be performed with fluorescent dyes such as Invitrogen's FM4-64 and FM1-43. With these fluorophores, we have been able to image plasma membrane and endomembrane compartments, as well as nectary morphology as a whole. It is expected that observations made via LSCM might help tease apart the general pathways of nectar secretion by providing in vivo analysis of nectary ultrastructure and organelle dynamics.

Molecular biology of nectaries

In addition to the transcription factors involved in nectary development described earlier, a wide variety of nectary-expressed genes have been identified, with putative functions ranging from sugar and starch metabolism (Ge et al. 2000; Ren et al. 2007b) to protecting nectar from microbial infection (Carter and Thornburg 2004; Kram et al. 2008; Peumans et al. 1997), among other functions (Nelson et al. 1997; Song et al. 2000; Tholl et al. 2005; Thoma et al. 1994). Despite the various reports on nectary-expressed genes, very little is known of the downstream mediators of nectary development, nectar synthesis, and secretion. Important work on petunia pollination syndromes has also identified a single QTL involved in controlling nectar volume and composition (Galliot et al. 2006a, b; Stuurman et al. 2004), though the specific gene(s) involved in mediating this phenomenon are currently unknown. While information provided through the reports described earlier is certainly valuable, a global picture of gene expression in nectaries has long been lacking.

The absence of genetic information describing nectar synthesis and secretion is rather astounding, especially considering the significant role nectaries play in the reproductive biology of many angiosperms. One of the hurdles has been finding a suitable organism in which to study nectary form and function—one that provides enough sample for analysis but that also has ample genetic resources available for rapid and functional analysis. To address the lack of a global picture of gene expression in nectaries, we recently demonstrated the feasibility of manually dissecting nectaries from Arabidopsis and then conducting transcriptomic studies (Kram et al. 2009). Still, this process is laborious, as Arabidopsis flowers, and hence nectaries, are very small. For each of the eight independent biological replicates used for these transcriptomic analyses,

approximately 200–300 nectaries, depending on nectary type, were required (~2,000 nectaries total). More specifically, the Affymetrix® ATH1 GeneChip array was used to follow global changes in gene expression in Arabidopsis nectaries at two developmental time points (pre-secretory and secretory nectaries), as well as between median and lateral nectaries. From these data, we were able to identify over 70 highly nectary-enriched genes (20 of which are listed in Table 1), with a significant subset being upregulated at specific floral developmental stages. It is hypothesized that these highly expressed and nectary-enriched genes are required for nectary development and/or function (e.g., see Fig. 3).

Logically, since sugars are the principal components of floral nectar, we expected carbohydrate metabolism genes to figure prominently in any list of nectary transcripts. In fact, upon microarray analysis, this was our finding, as the complete canonical sucrose biosynthetic pathway was upregulated in mature lateral nectaries when compared

with non-nectary reference tissues (Kram et al. 2009). As described earlier, various modifications appear to alter the composition of phloem sap to yield mature nectar (Fahn 1988). Nectaries are sink tissues, but specific biochemical and physiological processes are necessary to preserve this status and maintain the net flow of carbohydrates from source tissues into nectaries (Roitsch 1999; Sherson et al. 2003). Several enzymes (e.g., invertases, sucrose synthases, and sugar transporters) are capable of altering the carbohydrate composition of nectar. For example, sucrose and other disaccharides can be directly transported across a cell membrane (via transporters) into storage vacuoles, the cytosol, or the apoplast. Alternatively, cell wall invertases can hydrolyze sucrose into hexoses (glucose and fructose), which can then be imported by monosaccharide/proton symporters into sink cells. Indeed, Arabidopsis cell wall invertase genes were almost universally upregulated in mature lateral nectaries (compared with non-nectary reference tissues). *CELL WALL INVERTASE 4* (*AtCWINV4*,

Table 1 Some Arabidopsis genes displaying nectary-enriched expression profiles via microarray

Locus	TAIR annotation	Probe set	Fold difference over reference tissue avg ^a		
			ILN	MLN	MMN
AT2G30650	3-Hydroxyisobutyryl-coenzyme A hydrolase, putative	267571_at	234.44	381.26	274.91
AT4G12530	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	254829_at	316.88	376.33	352.76
AT3G25810	Myrcene/ocimene synthase, putative	257637_at	293.02	317.00	294.25
AT1G69180	Transcription factor CRC (CRABS CLAW)	260355_at	176.32	198.09	251.69
AT2G39060	Nodulin MtN3 family protein	266201_at	174.09	193.32	179.31
AT1G23300	MATE efflux family protein	263040_at	38.01	171.12	58.88
AT5G24270	Calcineurin B-like protein, putative/calcium sensor homolog (SOS3)	249783_at	127.08	132.06	151.92
AT1G77110	Auxin transport protein, putative	264962_at	52.91	84.81	90.87
AT1G17960	Threonyl-tRNA synthetase, putative/threonine-tRNA ligase, putative	255893_at	23.95	74.47	59.63
AT5G38130	Transferase family protein	249541_at	16.75	70.03	57.07
AT2G26580	Plant-specific transcription factor YABBY family protein	245029_at	43.81	68.71	64.28
AT2G36190	Beta-fructosidase, putative/beta-fructofuranosidase, putative	263905_at	65.77	66.63	62.00
AT5G42230	Serine carboxypeptidase S10 family protein	249241_at	0.71	53.38	34.80
AT2G22680	Zinc finger (C3HC4-type RING finger) family protein	265345_at	37.20	53.13	49.57
AT5G38120	4-Coumarate-CoA ligase family protein/4-coumaroyl-CoA synthase family protein	249540_at	38.12	51.34	55.60
AT2G44480	Glycosyl hydrolase family 1 protein	267391_at	1.06	50.02	18.52
AT1G23200	Pectinesterase family protein	264891_at	20.52	44.69	42.21
AT5G06720	Peroxidase, putative	250646_at	1.95	42.40	36.01
AT2G42830	Agamous-like MADS box protein AGL5/floral homeodomain transcription factor (AGL5)	263988_at	17.22	42.37	37.65
AT1G19640	S-Adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT)	261150_at	8.12	33.04	7.24

RNA isolated from manually collected Arabidopsis nectaries was labeled and then hybridized to the Affymetrix ATH1 GeneChip®. The resultant data were co-normalized with 13 non-nectary reference tissues and analyzed for nectary-enriched expression profiles (Kram et al. 2009)

^a Twenty genes displaying the highest nectary-to-reference tissue probe signal ratios are displayed. All genes had a minimum threefold higher probe signal intensity in nectaries than each individual reference tissue for at least one nectary type or developmental stage. ILN, immature lateral nectary (stage 11–12, pre-secretory); MLN, mature lateral nectary (stage 14–15, secretory); MMN, mature median nectary (stage 14–15, relatively non-secretory)

At2g36190), in particular, is strongly upregulated in Arabidopsis nectaries (see Table 1). Furthermore, *cwinv4* mutants fail to secrete nectar, which is presumably a result of disrupting the osmotic balance and sucrose gradient within nectaries (Ruhmann et al. submitted). Interestingly, an ortholog to *AtCWINV4* in *B. rapa*, *BrCWINV4*, also displays a nectary-specific expression profile. This suggests a conserved role for cell wall invertases in nectar secretion within the Brassicaceae, and perhaps even outside it.

In a previous study by Ge et al. (2000), the nectary-enriched gene *PhNEC1*, from *Petunia hybrida*, was implicated in nectar-carbohydrate metabolism and/or transport. We found the Arabidopsis ortholog to *PhNEC1*, nodulin MtN3 family protein (At2g39060), to be strongly expressed in the floral nectaries of Arabidopsis as well (Table 1). Furthermore, according to Ge et al. (2000), *Brassica napus* plants transformed with a *PhNEC1::GUS* construct showed a complementary nectary-enriched expression pattern that appeared to increase slightly with nectary development. Immunolocalization of PhNEC1 seemed to indicate fusion of small “NEC1-dotted” vesicles with the plasmalemma, coincident with nectar secretion. As described earlier, one proposed mechanism for nectar secretion is through fusion of vesicles with the plasma membrane (Ge et al. 2000); upon initial inspection, Ge et al. (2000) concluded that a granulocrine-type mechanism of nectar transport appears to be occurring in petunia. GUS expression also progressed from the nectary parenchyma of petunia (expressed here prior to anthesis and the onset of nectar secretion) to the epidermal cells once secretion had begun and was highest immediately after starch hydrolysis. During starch accumulation, GUS expression was limited both in intensity and distribution (Ge et al. 2000). Considering the suggested involvement of *PhNEC1* in sugar transport or metabolism, it will be very interesting to utilize the genetic tools available for Arabidopsis to confirm or invalidate this hypothesis.

Mining of the microarray data described earlier also suggested that several hormone biosynthesis and response pathways are upregulated in Arabidopsis nectaries, particularly those of auxin, jasmonic acid (JA), and gibberellins. This is significant, as some effects of these hormones on nectar production have been reported. For example, the production of free auxin (IAA) by nectaries begins immediately preceding anthesis and is a commonly occurring phenomenon amongst flowering plants (Endress 1994). This general occurrence was further documented in Arabidopsis by expression analyses of a known auxin-response element (DR5) fused to a GUS reporter construct, which illuminated the initiation and progression of free auxin production in nectary glands (Aloni et al. 2006). The findings by Aloni et al. (2006) indicate that free IAA serves two disparate functions in flower development—promoting

floral organ development in host organs, while repressing development in adjacent organs. Accordingly, anther-derived IAA in immature Arabidopsis flowers was suggested to limit nectar secretion until flower opening, and upon anthesis, nectaries become the sites of most free auxin synthesis in flowers (Aloni et al. 2006). Interestingly, the highest levels of free auxin production in rosette leaves occur in the hydathodes, which are principally water-secreting glands (Aloni et al. 2003). Hydathodes are believed to be the evolutionary precursors to extrafloral nectaries (Elias and Gelband 1977) and floral nectaries (Vogel 1998), all of which may share some similar mechanisms of secretion.

The reports described earlier are consistent with the finding that exogenous auxin application strongly reduced nectar secretion in cultured floral nectaries of *Euphorbia pulcherrima* and *Antirrhinum majus* (Matile 1956). Further work on excised snapdragon flowers generally supported a role for auxin in inhibiting nectar secretion while at the same time causing ovary enlargement, particularly at post-fertilization (Shuel 1959, 1964, 1978); however, in some instances, treatment with exogenous IAA resulted in an increase in nectar production, suggesting a dual-role for auxin in nectar production (Shuel 1964). Significantly, Shuel (1978) concluded that exogenously applied auxin impacts the secretory process itself, and not the movement of sugars to nectaries. In light of these findings, it should be noted that auxin is generally synthesized in localized tissues (e.g., meristems, nectaries) and then moved elsewhere via polarized fusion of secretory vesicles with the plasma membrane (Robert and Friml 2009; Weijers and Friml 2009). Thus, it is possible that the polar transport of auxin synthesized within nectaries may play a role in the directed secretion of nectar. Interestingly, an Arabidopsis auxin-efflux carrier family protein, *PIN6* (At1g77110), displayed nearly nectary-specific expression by microarray (Table 1; original data presented in Kram et al. 2009), and RT PCR and promoter::GUS analyses (Ruhmann et al. in preparation).

The effect of JA on nectar secretion by extrafloral nectaries can be pronounced; in one study on lima bean, secretion levels from the extrafloral nectaries of artificially wounded plants grown under controlled environmental settings reached 3,000% (on leaves) and 2,000% (on floral bracts) of control plant levels (Heil 2004). In separate field studies on *Macaranga tanarius*, increases of 200–500% in extrafloral nectary secretions were observed in response to wounding and exogenous JA treatment (Heil et al. 2001). Based upon anatomical and physiological similarities (Durkee 1982), as well as suggested evolutionary relatedness (De la Barrera and Nobel 2004), we hypothesize that JA-induced production of nectar might not be limited to extrafloral nectaries alone but might serve to modulate floral nectar production as well. In this line of thought, we

have identified a gene, *S*-Adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (*JMT*, At1g19640; Table 1), which is upregulated in all *Arabidopsis* nectaries, but most strongly in mature lateral nectaries (Kram et al. 2009). This gene product catalyzes the formation of methyljasmonate from jasmonic acid and is induced in response to wounding (Seo et al. 2001). *JMT* is the *Arabidopsis* ortholog of *BcNTRI*, a nectary-specific gene identified in *Brassica campestris* L. ssp. *pekinensis* (Song et al. 2000). In *Brassica campestris*, *NTRI* transcripts and protein are specific to the nectaries (both lateral and median). The temporal expression pattern of *NTRI* (mRNA levels are low in early flower development, increase during nectary development, and then rapidly decline following flower opening) support involvement of *NTRI* in nectary maturation or nectar production rather than nectary induction processes (Song et al. 2000).

Of note, in addition to its action in extrafloral nectaries and its involvement in wound response, JA is required for pollen maturation, anther dehiscence, and flower opening (Ishiguro et al. 2001). It is also well known that anther dehiscence and the initiation of nectar secretion are coincident (Schmid and Alpert 1977), and that several male-sterile homeotic mutants show defects in nectar production (Baum et al. 2001). Cumulatively, these results suggest a possible coordination of each of these events through JA-modulated pathways. One suggested mechanism for synchronization is through the involvement of JA in controlling water transport processes throughout the plant, or at the very least within and between specific floral tissues (Ishiguro et al. 2001). For example, accumulation of the proton-sucrose symporter, *AtSUC1*, in anther vasculature as this organ approaches its final developmental stages (Stadler et al. 1999) has led to speculation that JA might affect the expression of *AtSUC1*, as well as other genes that promote water flux throughout the stamen (Ishiguro et al. 2001). It is certainly tempting to hypothesize that similar JA-regulated processes might be operating in both extrafloral and floral nectaries to regulate water movement and, consequently, nectar secretion.

Similar to JA, exogenous treatment with the active gibberellic acid GA_3 has been reported to significantly increase nectar volume and sugar content in *Brassica* sp., resulting in a concomitant increase in pollinator visitation (Mishra and Sharma 1988). Significantly, gibberellin activity in *Arabidopsis* has been linked to the regulation of starch catabolism during seed germination (Pai et al. 1997). Potentially, gibberellins could serve a similar role in nectaries to regulate starch metabolism and maintain the sink status of nectaries, thus driving photosynthate transport into and out of the nectary. Despite the significance of the findings described earlier, it is important to note that the precise molecular mechanisms of JA, GA_3 , and auxin

involvement in nectar production are not currently understood.

Evaluation of nectar production in *Arabidopsis*

To study the impact of individual genes on nectar production, it is obviously necessary to observe and collect nectar secretions. Unfortunately, precise quantification of total nectar volume in *Arabidopsis* is difficult at best; however, because the nectar clings to the inner surface of sepals when gently peeled back (Fig. 3a), relative changes in total nectar volume can be determined. We have found this to be a crude, but consistent method for determining relative changes in *Arabidopsis* nectar volume. This analysis has been used to preliminarily identify altered secretion phenotypes in several nectary-specific gene mutants. For example, as described earlier, we have identified CELL WALL INVERTASE 4 (*AtCWINV4*) as an absolutely required factor for nectar production in *Arabidopsis*, as *cwinv4* T-DNA mutants do not secrete nectar (Fig. 3b) and also display altered starch accumulation patterns (Ruhlmann et al. submitted). Significantly, *Arabidopsis* can also be useful for studying aspects of nectary biology besides nectar production, including the generation of floral scents (Tholl et al. 2005), and hormonal impacts on floral development (Aloni et al. 2006).

Regarding nectar collection and compositional analysis, there is a single report of nectar being collected from *Arabidopsis* flowers via paper wicks (Davis et al. 1998). Although this study was performed *in vitro*, with the pedicels of mature floral buds placed in sucrose solution and incubated under very high humidity, Davis et al. (1998) were able to significantly increase the amount of nectar carbohydrate available for harvest and subsequent analysis in all three ecotypes investigated (e.g., Columbia—2.2x, Landsberg—2.8x, and Wassilewskija—2.5x), along with the actual volume of nectar. Under these conditions, the normally poorly producing median nectaries sometimes yielded sufficient volumes of nectar for compositional analysis. Since the hexose-dominant nature of *Arabidopsis* floral nectar was retained in samples collected from sucrose-cultured flowers, this suggests that the normal physiology of nectar production and secretion is being maintained under *in vitro* conditions. As an alternative to this method, we have determined that hand-drawn capillary pipettes can be used to collect nectar from freshly harvested flowers grown under standard conditions. While absolute volume is difficult to calculate—and highly dependent on developmental stage, soil moisture, physiology, and humidity—relative changes in volume between samples (e.g., wild-type vs. mutant) can be determined. Moreover, the nectar can be collected by expelling the fluid

into a microcentrifuge tube containing water. Initial studies to examine *Arabidopsis* nectar composition via non-targeted metabolomics analyses are currently underway.

Concluding remarks: why does *Arabidopsis* produce nectar?

Almost exclusively, *Arabidopsis thaliana* reproduces via self-pollination; yet, its flowers still contain functional and energetically demanding nectaries. To reconcile these seemingly incompatible findings, it is important to note that insects do indeed visit *Arabidopsis* flowers in their native setting, and that outcrossing occurs at low but discernable levels (Hoffmann et al. 2003). In general, closely related *Brassica* species do not share the autogamy of *Arabidopsis*, but instead are highly dependent upon insect pollinators to realize optimal reproductive success (Davis et al. 1996; Nieuwhof 1963; Pearson 1933; Rahman 1940; Vesely 1962). However, many Brassicaceae share similar nectarium structure with *Arabidopsis*, and produce relatively large volumes of nectar (Davis et al. 1996, 1998); these commonalities hint at the possibility of conserved machinery for nectary function within this plant family. Moreover, *Arabidopsis* nectary development appears to share common developmental mechanisms with much of the eudicot clade (Lee et al. 2005b).

Of course, determining the impacts of individual genes on nectary function within the context of reproductive success in would be a herculean undertaking. Thus, to complement the *Arabidopsis* studies described earlier, we have also produced 11,101 expressed sequence tags (ESTs) from *Brassica rapa* (oilseed rape) nectary cDNA libraries, which have been useful for identifying many orthologs to *Arabidopsis* nectary-enriched genes (Hampton et al. submitted). It is expected that *B. rapa* may be a useful parallel system for examining genetic impacts on nectar quality, pollinator visitation, and overall fecundity, with initial cues being taken from findings in *Arabidopsis*. Consequently, with all of the genetic information and genomic tools available, *Arabidopsis* provides unique advantages (coupled with several disadvantages) for studying nectary development and function at a molecular level, thereby retaining a high probability that findings will be widely applicable to flowering plants. With the above in mind, we have initiated large-scale studies to examine the mechanisms by which nectary-specific genes are involved in nectar synthesis and secretion throughout development.

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References

- Aloni R, Schwalm K, Langhans M, Ullrich CI (2003) Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in *Arabidopsis*. *Planta* 216:841–853
- Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of auxin in regulating *Arabidopsis* flower development. *Planta* 223:315–328
- Baker HG (1978) Chemical aspects of the pollination of woody plants in the tropics. In: Tomlinson PB, Zimmerman M (eds) *Tropical trees as living systems*. Cambridge University Press, New York, pp 57–82
- Baker H, Baker I (1973) Amino acids in nectar and their evolutionary significance. *Nature* 241:543–545
- Baker HG, Baker I (1982) Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In: Nitecki MH (ed) *Biochemical aspects of evolutionary biology*. University of Chicago Press, Chicago, pp 131–171
- Baker H, Baker I (1975) Studies of nectar-constitution and pollinator-plant coevolution. In: Gilbert LE, Raven PH (eds) *Coevolution of animals and plants*. University of Texas Press, Austin, pp 100–140
- Baker H, Baker I (1983) A brief historical review of chemistry of floral nectar. In: Bentley BL (ed) *The biology of nectaries*. Columbia University Press, New York, pp 126–152
- Baum SF, Eshed Y, Bowman JL (2001) The *Arabidopsis* nectary is an ABC-independent floral structure. *Development* 128:4657–4667
- Bernardello G (2007) A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and nectar*. Springer, Netherlands, pp 129–166
- Bowman JL (1994) *Arabidopsis: an atlas of morphology and development*. Springer-Verlag, New York
- Bowman JL, Smyth DR (1999) CRABS CLAW, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* 126:2387–2396
- Brandenburg A, Dell'olivo A, Bshary R, Kuhlemeier C (2009) The sweetest thing advances in nectar research. *Curr Opin Plant Biol* 12:1–5
- Carter C, Thornburg RW (2004) Is the nectar redox cycle a floral defense against microbial attack? *Trends Plant Sci* 9:320–324
- Carter C, Graham RA, Thornburg RW (1999) Nectarin I is a novel, soluble germin-like protein expressed in the nectar of *Nicotiana* sp. *Plant Mol Biol* 41:207–216
- Cutler SR, Ehrhardt DW, Griffiths JS, Somerville CR (2000) Random GFP:cDNA fusions enable visualization of subcellular structures in cells of *Arabidopsis* at a high frequency. *Proc Natl Acad Sci USA* 97:3718–3723
- Davis AR (2001) Searching and breeding for structural features of flowers correlated with high nectar-carbohydrate production. *Acta Hort* 561:107–121
- Davis A, Peterson R, Shuel R (1986) Anatomy and vasculature of the floral nectaries of *Brassica napus* (Brassicaceae). *Can J Bot* 64:2508–2516
- Davis AR, Fowke LC, Sawhney VK, Low NH (1996) Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae) II. Quantified variability of nectary structure and function in rapid-cycling lines. *Ann Bot* 77:223–234
- Davis AR, Pylatuik JD, Paradis JC, Low NH (1998) Nectar-carbohydrate production and composition vary in relation to

- nectary anatomy and location within individual flowers of several species of Brassicaceae. *Planta* 205:305–318
- De la Barrera E, Nobel PS (2004) Nectar: properties, floral aspects, and speculations on origin. *Trends Plant Sci* 9:65–69
- Deeken R, Geiger D, Fromm J, Koroleva O, Ache P, Langenfeld-Heysler R, Sauer N, May ST, Hedrich R (2002) Loss of the AKT2/3 potassium channel affects sugar loading into the phloem of *Arabidopsis*. *Planta* 216:334–344
- Deinzer ML, Thomson PA, Burgett DM, Isaacson DL (1977) Pyrrolizidine alkaloids: their occurrence in honey from tansy ragwort (*Senecio jacobaea* L.). *Science* 195:497–499
- Di Sansebastiano GP, Paris N, Marc-Martin S, Neuhaus JM (2001) Regeneration of a lytic central vacuole and of neutral peripheral vacuoles can be visualized by green fluorescent proteins targeted to either type of vacuoles. *Plant Physiol* 126:78–86
- Durkee LT (1982) The floral and extra-floral nectaries of *Passiflora*. II. The extra-floral nectary. *Am J Bot* 69:1420–1428
- Durkee LT (1983) The ultrastructure of floral and extrafloral nectaries. In: Bentley B, Elias T (eds) *The biology of nectaries*. Columbia University Press, New York, pp 1–29
- Ecroyd CE, Franich RA, Kroese HW, Steward D (1995) Volatile constituents of *Cactylanthus taylorii* flower nectar in relation to flower pollination and browsing by animals. *Phytochemistry* 40:1387–1389
- Elias T, Gelband H (1977) Morphology, anatomy, and relationship of extrafloral nectaries and hydathodes in two species of Impatiens (Balsaminaceae). *Botanical Gazette* 138:206–212
- Endress P (1994) *Diversity and evolutionary biology of tropical flowers*. Cambridge University Press, Cambridge
- Fahn A (1979a) *Secretory tissues in plants*. Academic Press, London
- Fahn A (1979b) Ultrastructure of nectaries in relation to nectar secretion. *Am J Bot* 66:977–985
- Fahn A (1988) Tansley review No. 14 secretory tissues in vascular plants. *New Phytol* 108:229–257
- Fallahi H, Scofield GN, Badger MR, Chow WS, Furbank RT, Ruan YL (2008) Localization of sucrose synthase in developing seed and siliques of *Arabidopsis thaliana* reveals diverse roles for SUS during development. *J Exp Bot* 59:3283–3295
- Ferrerres F, Andrade P, Gil MI, Tomas Barberan FA (1996) Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. *Z Lebensm Unters Forsch* 202:40–44
- Galliot C, Hoballah ME, Kuhlemeier C, Stuurman J (2006a) Genetics of flower size and nectar volume in *Petunia* pollination syndromes. *Planta* 225:203–212
- Galliot C, Stuurman J, Kuhlemeier C (2006b) The genetic dissection of floral pollination syndromes. *Curr Opin Plant Biol* 9:78–82
- Ge YX, Angenent GC, Wittich PE, Peters J, Franken J, Busscher M, Zhang LM, Dahlhaus E, Kater MM, Wullems GJ, Creemers-Molenaar T (2000) NEC1, a novel gene, highly expressed in nectary tissue of *Petunia hybrida*. *Plant J* 24:725–734
- Grebe M, Xu J, Mobius W, Ueda T, Nakano A, Geuze HJ, Rook MB, Scheres B (2003) *Arabidopsis* sterol endocytosis involves actin-mediated trafficking via ARA6-positive early endosomes. *Curr Biol* 13:1378–1387
- Griebel C, Hess G (1990) The vitamin C content of flower nectar of certain Labiatae. *Z Unters Lebensm* 79:168–171
- Heil M (2004) Induction of two indirect defences benefits Lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *J Ecol* 92:527–536
- Heil M, Koch T, Hilpert A, Fiala B, Boland W, Linsenmair K (2001) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proc Natl Acad Sci USA* 98:1083–1088
- Heinrich G (1989) Analysis of cations in nectars by means of a laser microprobe mass analyser (LAMMA). *Beitr Biol Pflanz* 64:293–308
- Hoffmann MH, Bremer M, Schneider K, Burger F, Stolle E, Moritz G (2003) Flower visitors in a natural population of *Arabidopsis thaliana*. *Plant Biol* 5:491–494
- Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The DEFECTIVE IN ANTHHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. *Plant Cell* 13:2191–2209
- Jolivet P (1992) *Insects and plants: parallel evolution & adaptations*. CRC Press, Boca Raton
- Kram BW, Bainbridge EA, Perera MADN, Carter C (2008) Identification, cloning and characterization of a GDSL lipase secreted into the nectar of *Jacaranda mimosifolia*. *Plant Mol Biol* 68:173–183
- Kram BW, Xu WW, Carter CJ (2009) Uncovering the *Arabidopsis thaliana* nectary transcriptome: investigation of differential gene expression in floral nectariferous tissues. *BMC Plant Biol* 9:92
- Lee JY, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL (2005a) Activation of CRABS CLAW in the nectaries and carpels of *Arabidopsis*. *Plant Cell* 17:25–36
- Lee JY, Baum SF, Oh SH, Jiang CZ, Chen JC, Bowman JL (2005b) Recruitment of CRABS CLAW to promote nectary development within the eudicot clade. *Development* 132:5021–5032
- Matile P (1956) On the metabolism and the auxin dependence of nectar secretion. *Berichte der Schweizerischen Botanischen Gesellschaft* 66:237–266
- McKim SM, Stenvik GE, Butenko MA, Kristiansen W, Cho SK, Hepworth SR, Aalen RB, Haughn GW (2008) The BLADE-ON-PETIOLE genes are essential for abscission zone formation in *Arabidopsis*. *Development* 135:1537–1546
- Mishra R, Sharma S (1988) Growth regulators affect nectar-pollen production and insect foraging in Brassica seed crops. *Curr Sci India* 57:1297–1299
- Nelson DE, Glaunsinger B, Bohnert HJ (1997) Abundant accumulation of the calcium-binding molecular chaperone calreticulin in specific floral tissues of *Arabidopsis thaliana*. *Plant Physiol* 114:29–37
- Nepi M (2007) Nectary structure and ultrastructure. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and nectar*. Springer, Dordrecht, pp 129–166
- Nepi M, Stpiczynska M (2008) The complexity of nectar: secretion and resorption dynamically regulate nectar features. *Naturwissenschaften* 95:177–184
- Nieuwhof M (1963) Pollination and contamination of *Brassica oleracea* L. *Euphytica* 12:17–26
- Nieuwhof M (1969) *Cole crops*. Leonard Hill, London
- Pacini E, Nepi M (2007) Nectar production and presentation. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and nectar*. Springer, Dordrecht, pp 167–214
- Pai H, Mariani C, Kao T (1997) Cytological study of pollen tube growth and early seed development in *Petunia inflata*. *J Plant Biol* 40:212–219
- Pearson OH (1933) Study of the life history of *Brassica oleracea*. *Bot Gaz* 94:534–550
- Peng YB, Li YQ, Hao YJ, Xu ZH, Bai SN (2004) Nectar production and transportation in the nectaries of the female *Cucumis sativus* L. flower during anthesis. *Protoplasma* 224:71–78
- Peumans WJ, Smeets K, Van Nerum K, Van Leuven F, Van Damme EJ (1997) Lectin and alliinase are the predominant proteins in nectar from leek (*Allium porrum* L.) flowers. *Planta* 201:298–302
- Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Curr Opin Plant Biol* 5:237–243

- Rahman KA (1940) Insect pollinators of toria (*Brassica napus* Linn., var. *dichotoma* prain) and sarson (*B. campestris* Linn., var. *sarson* prain) at Lyallpur. Indian J Agr Sci 10:422–447
- Rathman ES, Lanza J, Wilson J (1990) Feeding preferences of flesh flies (*Sarcophaga bullata*) for sugar-only vs. sugar-amino acid nectars. Am Midl Nat 124:379–389
- Ren G, Healy RA, Klyne AM, Horner HT, James MG, Thornburg RW (2007a) Transient starch metabolism in ornamental tobacco floral nectaries regulates nectar composition and release. Plant Sci 173:277–290
- Ren G, Healy RA, Horner HT, Martha GJ, Thornburg RW (2007b) Expression of starch metabolic genes in the developing nectaries of ornamental tobacco plants. Plant Sci 173:621–637
- Robert HS, Friml J (2009) Auxin and other signals on the move in plants. Nature Chemical Biology 5:325–332
- Roitsch T (1999) Source-sink regulation by sugar and stress. Curr Opin Plant Biol 2:198–206
- Roshchina VV, Roshchina VD (1993) The excretory function of higher plants. Springer-Verlag, New York
- Rusterholz HP, Erhardt A (2000) Can nectar properties explain sex-specific flower preferences in the Adonis blue butterfly *Lysandra bellargus*? Ecol Entomol 25:81–90
- Schmid R, Alpert PH (1977) A test of Burk's hypothesis relating anther dehiscence to nectar secretion. New Phytol 78:487–498
- Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci USA 98:4788–4793
- Sherson SM, Alford HL, Forbes SM, Wallace G, Smith SM (2003) Roles of cell-wall invertases and monosaccharide transporters in the growth and development of Arabidopsis. J Exp Bot 54:525–531
- Shuel RW (1959) Studies of nectar secretion in excised flowers. II. The influence of certain growth regulators and enzyme inhibitors. Can J Bot 37:1167–1180
- Shuel RW (1964) Nectar secretion in excised flowers. III. The dual effect of indolyl-3-acetic acid. J Apicult Res 3:99–111
- Shuel RW (1978) Nectar secretion in excised flowers. V. Effects of indoleacetic acid and sugar supply on distribution of [¹⁴C]sucrose in flower tissues and nectar. Can J Bot 56:565–571
- Smyth DR, Bowman JL, Meyerowitz EM (1990) Early flower development in Arabidopsis. Plant Cell 2:755–767
- Song JT, Seo HS, Song SI, Lee JS, Choi YD (2000) NTR1 encodes a floral nectary-specific gene in *Brassica campestris* L. ssp. *pekinensis*. Plant Mol Biol 42:647–655
- Stadler R, Truernit E, Gahrz M, Sauer N (1999) The AtSUC1 sucrose carrier may represent the osmotic driving force for anther dehiscence and pollen tube growth in Arabidopsis. Plant J. 19:269–278
- Stuurman J, Hoballah ME, Broger L, Moore J, Basten C, Kuhlemeier C (2004) Dissection of floral pollination syndromes in petunia. Genetics 168:1585–1599
- Tholl D, Chen F, Petri J, Gershenzon J, Pichersky E (2005) Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from Arabidopsis flowers. Plant J 42:757–771
- Thoma S, Hecht U, Kippers A, Botella J, Devries S, Somerville C (1994) Tissue-specific expression of a gene encoding a cell wall-localized lipid transfer protein from Arabidopsis. Plant Physiol 105:35–45
- Tian GW, Mohanty A, Chary SN, Li S, Paap B, Drakakaki G, Kopec CD, Li J, Ehrhardt D, Jackson D, Rhee SY, Raikhel NV, Citovsky V (2004) High-throughput fluorescent tagging of full-length Arabidopsis gene products in planta. Plant Physiol 135:25–38
- Vesely V (1962) The economic effectiveness of bee pollination on winter rape (*Brassica napus* L., var. *oleifera* metz.). Min Zemedel Lesn a Vodniho Hospodar Ust Vedtach Inform Zemedel Ekon 8:659–673
- Vogel S (1969) Flowers offering fatty oil instead of nectar. Abstracts XIth International Botany Congress Seattle, WA
- Vogel S (1998) Remarkable nectaries: structure, ecology, organophyletic perspectives IV. Miscellaneous cases. Flora 193:225–248
- Wang YS, Motes CM, Mohamalawari DR, Blancaflor EB (2004) Green fluorescent protein fusions to Arabidopsis fimbrin 1 for spatio-temporal imaging of F-actin dynamics in roots. Cell Motil Cytoskeleton 59:79–93
- Weber LG (1958) Nutrition and reproduction in the Australian sheep blowfly *Lucilia cuprina*. Aust J Zool 6:139–144
- Weijers D, Friml J (2009) Snapshot: auxin signaling and transport. Cell 136:U1172–U1200
- Wenzler M, Holscher D, Oerther T, Schneider B (2008) Nectar formation and floral nectary anatomy of *Anigozanthos flavidus*: a combined magnetic resonance imaging and spectroscopy study. J Exp Bot 59:3425–3434
- Wist TJ, Davis AR (2006) Floral nectar production and nectary anatomy and ultrastructure of *Echinacea purpurea* (Asteraceae). Ann Bot 97:177–193
- Wist TJ, Davis AR (2008) Floral structure and dynamics of nectar production in *Echinacea pallida* var. *angustifolia* (Asteraceae). Int J Plant Sci 169:708–722
- Zhu J, Hu ZH (2002) Cytological studies on the development of sieve element and floral nectary tissue in *Arabidopsis thaliana*. Acta Bot Sin 44:9–14
- Zhu J, Hu Z, Muuml IM (1995) Ultrastructural investigations on floral nectary of *Arabidopsis thaliana* prepared by high pressure freezing and freeze substitution. Biol Cell 84:225
- Zhu J, Hu ZH, Müller M (1997) Ultrastructure of the floral nectary of *Arabidopsis thaliana* L. prepared from high pressure freezing and freeze substitution. Acta Bot Sin 39:289–295