Trends in
# Plant Science

Volume 22 Number 4
April 2017
ISSN 1360-1385

## The Floral Metabolic Network that Produces Signals for Pollinators

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Review

# The Sexual Advantage of Looking, Smelling, and Tasting Good: The Metabolic Network that Produces Signals for Pollinators

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**A striking feature of the angiosperms that use animals as pollen carriers to sexually reproduce is the great diversity of their flowers with regard to morphology and traits such as color, odor, and nectar. These traits are underpinned by the synthesis of secondary metabolites such as pigments and volatiles, as well as carbohydrates and amino acids, which are used by plants to lure and reward animal pollinators. We review here the knowledge of the metabolic network that supports the biosynthesis of these compounds and the behavioral responses that these molecules elicit in the animal pollinators. Such knowledge provides us with a deeper insight into the ecology and evolution of plant-pollinator interactions, and should help us to better manage these ecologically essential interactions in agricultural ecosystems.**

## Trends

Novel routes for the biosynthesis of floral volatiles in unusual subcellular compartments are being identified and new theories on the emission of scent are proposed.

Key genes in betalain biosynthesis have been identified and new theories on the evolutionary origin of the pathway have been suggested.

Master regulators that coordinately control the production of pigments and scent in flowers are emerging.

Loci have been identified that contribute to reproductive isolation by pollination preferences for visual and olfactory cues and ultimately lead to speciation.

## Flower Signals in The Communication Between Plants and Their Pollinators

Outcrossing plants that rely on biotic pollination to sexually reproduce signal the presence of **floral rewards** (see Glossary) to animal pollen vectors with their smell and color, and reward these visits with nectar and pollen. Color, odor, and the composition of nectar and pollen are the phenotypic display of the metabolic resources of flowers that plants use in this **chemical and visual communication** with their pollinators. Recent discoveries have identified new pathways for the synthesis of floral metabolites, and new hypotheses have been formulated concerning the accumulation of pigments and release of fragrance. Concomitantly, advances in the field of plant–insect communication have disentangled the contribution of individual **floral signals** in attracting pollinators. In this review we discuss the recent discoveries on the biosynthesis, emission, and accumulation of floral metabolites of relevance for attracting and rewarding pollinators, as well as their ecological and evolutionary relevance. We limit our discussion to the most recent discoveries and refer to earlier reviews for a broader overview on specific topics. Finally, we discuss new opportunities and as yet unsolved biological questions that have arisen from these discoveries.

## Flower Color

Secondary metabolites of the families of flavonoids, carotenoids, and betalains collectively known as floral pigments produce the typical hues and color patterns observed in the showy

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 http://dx.doi.org/10.1016/j.tplants.2016.12.009

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**perianth** and pollen of flowers. We discuss these on a compound-class basis below and provide a schematic overview of the sites of their cellular biosynthesis and storage in Figure 1.

Flavonoids (Figure 2A) are the pigments that contribute to the development of the widest spectrum of flower colors. Among these, flavones, flavonols, and flavanones produce colors that vary from white to creamy, chalcones and aurones for shades of yellow, and anthocyanins for pink, red, and blue hues [1]. From a biochemical perspective, the flavonoid biosynthetic

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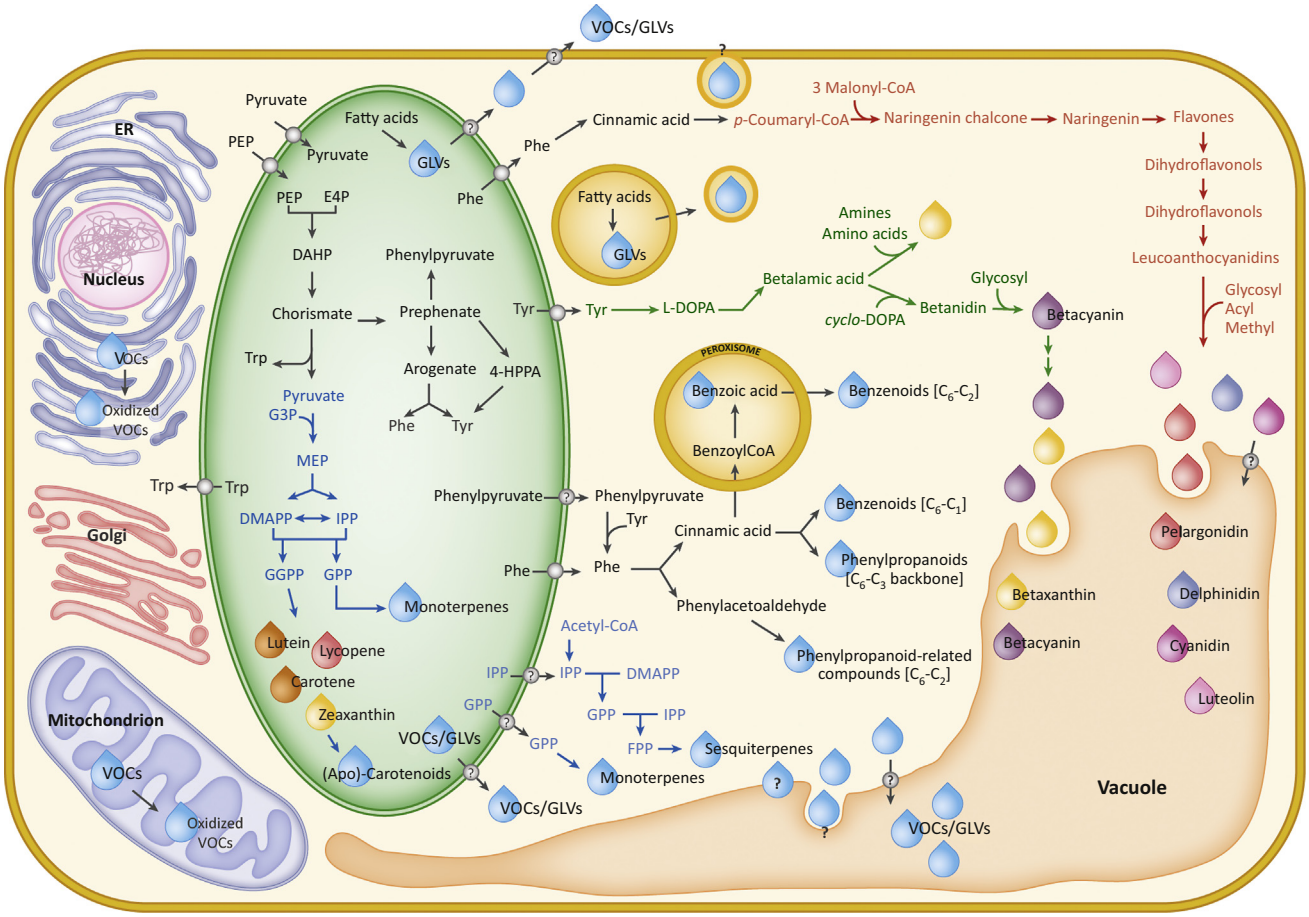


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Figure 1. Biosynthetic Pathways of Pigments, Volatile Organic Compounds (VOCs), and Green Leaf Volatiles (GLVs) and Their Sites of Subcellular Storage and Emission. The biosynthesis of Phe, Tyr, and terpenes takes place in the plastid (green oval) through the chorismate/prephenate (pathway in black) and the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway (in blue), respectively [1,20,28–31]. Red, orange, and yellow droplets inside the plastid symbolize carotenoid pigments (lycopene, carotene, lutein, zeaxanthin). Droplets colored in light blue represent terpenes, apocarotenoids, and GLVs, the latter being produced from the degradation of fatty acids. Grey circles represent carriers and/or transporters and putative (labeled with a question mark) transporters of metabolites across the membrane of the plastid. In the cytosol, the biosynthesis of flavonoids (pathway in red) from Phe produces the pigments pelargonidin, delphinidin, and cyanidin/luteolin that are shown as red, blue, and purple droplets, respectively [3]. Betaxanthin (yellow droplets) and betacyanin (purple droplets) are synthesized from cytosolic Tyr through the betalain biosynthetic pathway (in green) [16–18]. After glycosylation, acylation, and methylation, the pigments are transported from the cytosol into the vacuole via a process of microautophagy [4] and/or by putative carriers (represented with grey circles with a question mark). In the cytosol, Phe and phenylpyruvate contribute to the biosynthesis of volatile benzenoids, phenylpropanoids, and phenylpropanoid-related compounds. Other VOCs produced in the cytosol are synthesized via the mevalonate (MVA) pathway (in blue) [20]. Terpenes produced in the plastid or cytosol are further modified in the mitochondrion and ER [22,23]. In the peroxisomes, VOCs of the class of benzenoids are produced from cinnamic acid, whereas GLVs derive from the degradation of fatty acids. The existence of specific carriers and/or transporters for VOCs and GLVs emission has been postulated, but not yet demonstrated [42]. It is presumed that the vacuole serves as a site for subcellular storage of VOCs and GLVs, but this has not been demonstrated. Abbreviations: DAHP, 3-deoxy-D-arabinoheptulosonate 7-phosphate; DMAPP, dimethylallyl pyrophosphate; DOPA, dihydroxyphenylalanine; E4P, erythrose 4-phosphate; ER, endoplasmic reticulum; FPP, farnesyl pyrophosphate; G3P, glyceraldehyde 3-phosphate; GPP, geranyl pyrophosphate; 4-HPPA, 4-hydroxyphenylpyruvic acid; IPP, isopentenyl pyrophosphate; PEP, phosphoenolpyruvate.

pathway (Figure 1, pathway in red) links the phenylpropanoid and polyketide pathways which provide the *p*-coumaryl-CoA and malonyl-CoA units for the condensation reaction catalyzed by CHALCONE SYNTHASE to form the precursor of all flavonoid molecules, naringenin chalcone. Subsequent isomerization of this tetrahydrochalcone by CHALCONE ISOMERASE (CHI) closes the C-ring to form naringenin, which is then further modified by a range of oxidation and reduction reactions. Following their synthesis in the cytosol, flavonoids are modified by glycosylation, methylation, and acylation, and they subsequently accumulate in the vacuole [2,3].

The physical separation of flavonoid biosynthesis in the cytosol and accumulation in the vacuole has been known for a long time. However, the mechanism of transport between these two compartments remained unclear until recently. Petals of *Eustoma grandiflorum* of the Gentianaceae family (commonly known as lisianthus) that accumulate purple agglomerates of anthocyanin vacuolar inclusions (AVI) were used to reveal that the pigments aggregate in the cytosol and are subsequently engulfed by the vacuole via a process of microautophagy that had not been observed in plants before [4]. Similarly, in *Arabidopsis thaliana* it was shown that the physical vicinity of anthocyanin clumps to the external side of the tonoplast is sufficient to mediate the engulfment and release of pigments into the lumen of the vacuole, and that the process is independent from autophagy-related protein 5 (ATG5) and the endoplasmic reticulum (ER) [5]. In addition to this fascinating mechanism, several specific tonoplast transporter proteins for anthocyanins have been characterized or at least putatively identified [6], suggesting that the regulation of sequestration of anthocyanins and other flavonoids in the vacuole is highly complex.

The accumulation of flavonoids in the vacuole is very important for the color of flowers. Variations in the pH of the vacuole induce changes in the redox state of the flavonoid molecules which causes a shift in the spectrum of light absorbance that affects the hue of petals. Anthocyanins, for example, are red at low pH and blue at high pH [7]. Therefore, proton and ion transporters that create and maintain the acidity of the vacuolar lumen directly contribute to flower color [8,9]. Degradation of floral pigments takes also place in the vacuole in a tightly regulated developmental process. For example, a burst of vacuolar class III peroxidase activity results in a rapid color change from deep purple to white in *Brunfelsia calycina* flowers, which is why the name 'yesterday, today, tomorrow' *Brunfelsia* was given to the plant [10,11]. Upon flower fertilization, vacuolar degradation of pigments may be induced, presumably to discourage flower visitation by pollinators and recycle carbon to the fruits and seeds that soon will start developing [12].

The second largest class of floral pigments are the carotenoids (Figure 2B), which are lipophilic isoprenoid compounds that accumulate in petals and pollen, and confer yellow and red colors to the flowers. Moreover, because carotenoids can coexist in the same tissues with flavonoids or betalains, they also contribute to the development of the hues of brown and gold [1]. The biosynthesis of carotenoids takes place in the plastids, where two molecules of geranylgeranyl diphosphate (GGPP) are condensed to form phytoene (Figure 2B), which is then converted to *trans*-lycopene by desaturation and isomerization reactions. Through the activity of ε- and β-cyclases or two β-cyclases, lycopene is subsequently converted to α-carotene or β-carotene, respectively, starting from which the biosynthesis of carotenoids proceeds along two separate branches. In flowers, carotenoids are stored in chromoplasts or oxidatively cleaved into apocarotenoids, some of which are volatile and contribute to the scent of flowers (synthesis of apocarotenoids is described in the section Post-Transcriptional Trade-Off Between Color and Odor).

Finally, betalains are nitrogen-containing pigments found in plant species of the Caryophyllales and whose presence is mutually exclusive with that of anthocyanins [13]. The chromophore and

## Glossary

**Associative learning:** a neural process by which pollinators associate different stimuli, for example color or scent, with the presence of a reward.
**Bull's-eye:** a color pattern where the center and the margin of the flower are concentric circles of different colors.
**Chemical and visual communication:** exchange of information via chemical and visual signals.
**Enantiomer:** molecules that are 3D mirror images of one another.
**Floral mimicry:** the imitation of a rewarding 'model' by a mimetic flower, often without the production of a reward.
**Floral rewards:** metabolites (mostly sugars and amino acids, present in nectar) and proteins (pollen) present in a flower that are attractive for pollinators.
**Floral signals:** volatiles or color (or other traits) that have evolved to be detected by a signal receiver (pollinator).
**Nectarguides:** color or odor patterns on the perianth that direct pollinators to the nectar.
**Perianth:** non-reproductive part of a flower, comprises petals and sepals.
**Picotee:** color pattern where the distal margin of the petals have a different color.
**Phenotypic plasticity:** the change in phenotype in response to an environmental factor.
**Pollination syndrome:** convergent evolution of similar flower traits (morphology, color, scent, nectar) in response to selection imposed by shared pollinators.
**Positive directional selection:** stronger expression of a particular trait (for example large flowers) that is associated with higher fitness (e.g., higher seed production).
**Venation:** thin strips of color on the petals.

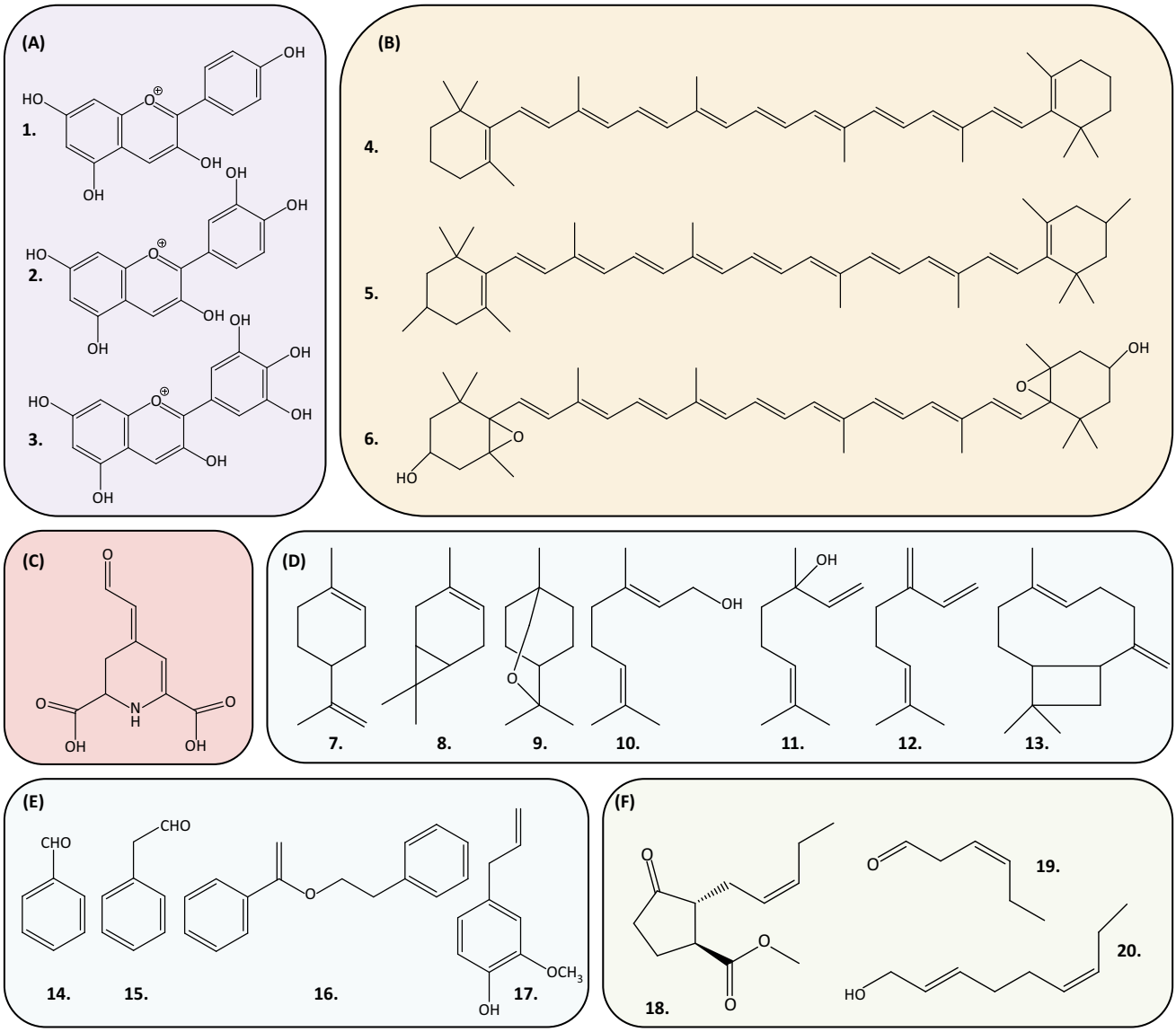


Figure 2. Examples of Structures of Pigments and Volatile Organic Compounds (VOCs) Produced in Flowers. (A) anthocyanidins: 1, pelargonidin; 2, cyanidin; 3. delphinidin; (B) carotenoids: 4, β-carotene; 5, zeaxanthin; 6, violaxanthin; (C) betalamic acid; (D) terpenoids: 7, limonene; 8, car-3-ene; 9, 1,8-cineole; 10, geraniol; 11, linalool; 12, myrcene; 13, (*E*)-β-caryophyllene; (E) benzenoids and phenylpropanoids: 14, benzaldehyde; 15, phenylacetaldehyde; 16, phenylethylbenzoate; 17, eugenol; (F) green leaf volatiles: 18, methyl jasmonate; 19, (*Z*)-3-hexenal; 20, (*E,Z*)-2,6-nonadienol.

central metabolite of the betalain biosynthetic pathway (Figure 1, pathway in green) is betalamic acid (Figure 2C), which is produced via oxidization of the amino acid tyrosine (Tyr). Once betalamic acid is formed, it spontaneously reacts with amines and amino acids to form betaxanthins, which are yellow. Alternatively, betalamic acid reacts with *cyclo*-DOPA to form betanidin, the aglycone moiety of the violet betacyanins. Glycosylation of betanidin results in the formation of betalains, which subsequently accumulate in the vacuole. Interestingly, orthologs of the same genes and enzymes that decorate the anthocyanins are responsible for hydroxylating betalains in the Caryophyllales [14]. In addition, the *cyclo*-DOPA moiety can be glycosylated, which gives rise to a different set of colors, as for example the red pigmentation of the

### Box 1. The Synthesis of Floral Metabolites Is Coordinately Regulated

Pollination signals are produced during the day or night depending on when pollinators forage for nectar and pollen, and their biosynthesis is primarily regulated by the circadian clock [98]. For example, in flowers of white *Petunia* varieties which are nocturnally pollinated by moths, LATE ELONGATED HYPOCOTYL (LHY [99]) and ODORANT1 (ODO1 [100]) are the master regulators of temporal emission of benzenoids. At night, ODO1 transcriptionally activates the genes of the shikimate pathway, leading to increased production of the precursors of volatile compounds, while LHY transcriptionally represses those genes during the daytime. Transcriptional regulators of the flavonoid pathway have also been characterized in depth [101–103], and color patterns, such as dots, segments (**picotee**) and stripes (**venation**), which guide pollinators towards nectar or pollen rewards have recently been identified. For example, in the bumblebee pollinated *Mimulus lewisii*, which bears white flowers with a pink marginal picotee, the R2R3-MYB transcription factor LIGHT AREAS 1 (LAR1) positively regulates *FLAVONOL SYNTHASE* (*FLS*) which redirects flavonoid biosynthesis from the pink colored anthocyanidins towards the formation of colorless flavonols. Instead, in *Mimulus cardinalis* where *LAR1* is poorly expressed, the corolla accumulates red anthocyanins that attract hummingbird pollinators [104]. Veins of color on the corolla (venation) that serve as nectarguides confer a pollination advantage to the flowers [105,106], and their formation is also transcriptionally regulated. *VENOSA* in *Antirrhinum majus* [106] and *DEEP PURPLE* (*DPL*) in *Petunia hybrida* [107] encode members of the R2R3-MYB family of transcription factors that are involved in this regulatory process. Finally, the biosynthesis of betalains is also regulated by a R2R3 MYB-like transcription factor which has been coopted from the anthocyanin pathway to regulate pigment accumulation in species of the Caryophylalles [17]. There is also evidence that the biosynthesis of pigments and volatiles is regulated by common transcription factors. For example, *PRODUCTION OF ANTHOCYANIN PIGMENT1* (*PAP1*) from *Arabidopsis thaliana* coregulates the accumulation of anthocyanins and emission of volatiles when expressed in transgenic *Petunia hybrida* and *Rosa hybrida* [108,109]. Similarly, the MYB-R2R3 transcription factor PH4 that controls petal pigmentation via acidification of the vacuole (from which the name PH is derived [110]) regulates the production of the internal pool of phenylpropanoids in addition to color [111]. Based on these observations, it is hypothesized that additional common switches that coordinately regulate color and odor must exist in flowers [111], while other studies provide evidence for the (coordinated) upregulation of primary metabolism to support this enhanced investment in the production of secondary metabolites such as pigments [112–114].

perianth in *Mirabilis jalapa* [15]. It was known since the beginning of the 1900 that the production of betalains is linked to the *R* and *Y* loci that control red versus yellow (R) and the presence versus absence (Y) of color, respectively. The lack of betalain production in model organisms has delayed the identification of the underlying genes. It is now known that *BvCYP76AD1* at the *R* locus encodes a cytochrome P450 enzyme that mediates the oxidation of L-DOPA to *cyclo*-DOPA quinone [16], and that a MYB-like transcription factor encoded at the *Y* locus (*BvMYB1*) regulates the appearance of color [17]. More recently, other genes encoding key enzymes in the betalain pathway were identified [13,18], as well as the transposon mutation that causes the red and yellow variegation of the perianth in *Mirabilis jalapa* [19]. Regulatory genes of pigment biosynthesis are discussed in Box 1.

## Floral Scent

Floral scent consists of a blend of molecules of low molecular weight and high vapor pressure that diffuse into the environment to signal the position of the flowers to pollinators. The major classes of volatiles emitted from flowers are terpenoids, benzenoids, and phenylpropanoids (derived from phenylalanine, Phe), and fatty acid-derived green leaf volatiles (GLVs) [20].

Terpenoids (Figure 2D) represent the largest and most diverse class of flower volatiles. Their biosynthesis is catalyzed by terpene synthases (TPSs) that convert prenyl substrates, which derive from units of isopentenyl diphosphate (IPP; Figure 2D), such as geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and GGPP into cyclic and acyclic terpenes [21]. Two major routes contribute to the biosynthesis of these prenyl precursors: the methylerythritol phosphate (MEP) pathway, which is active in the plastids, and the mevalonic acid (MVA) pathway (Figure 1, pathways in blue), which operates in the cytosol and peroxisomes [22]. It is generally accepted that there is a physical separation between the sites of terpene biosynthesis within the cell: monoterpenes and diterpenes are primarily produced in the plastids, whereas sesquiterpenes and triterpenes are primarily present in the cytosol. However, secondary modifications to the chemical structure of terpenes also take place in mitochondria, ER, and peroxisomes [23]. A less thoroughly investigated pathway for the biosynthesis of terpenes is that mediated by the

activity of cellular phosphatases. Despite the fact that geraniol synthases (which are TPSs) have been cloned from many plant species, a study conducted in the early 1980s had already suggested that terpene alcohols such as geraniol, nerol, and farnesol could be produced from GPP and FPP by the activity of phosphatases [24]. The genetic and biochemical evidence that this alternative biosynthetic route actually occurred *in planta* was only recently published. The identification of RhNUDX1, the enzyme responsible for the production of geraniol in rose petals, and the demonstration that RhNUDX1 hydrolyzes GPP to GP, which is further converted to geraniol by an as yet uncharacterized cellular phosphatase, provided such evidence [25]. Intriguingly, RhNUDX1 is active in the cytosol, an unusual subcellular compartment for the synthesis of monoterpenes, which generally takes place in the plastids. The driving force that resulted in the evolution of this alternative geraniol production system in cultivated rose, and the origin of GPP used by RhNUDX1 to synthesize geraniol, remain an enigma. Exchange of IPP and GPP between subcellular organelles has been indirectly suggested in many studies [26,27]. A transporter is probably involved in this exchange but has not yet been identified.

Another class of volatile compounds emitted from flowers are the derivatives of the amino acid Phe (Figure 2E), which is primarily synthesized in the plastids through the arogenate pathway (Figure 1, pathway in black) and further modified by enzymes present in the cytosol and peroxisomes [28]. Flowers of *Petunia hybrida* that produce and release large amounts of Phe-derived volatiles and benzenoids served as a model to study the biosynthesis of Phe. In these studies the enzymes PREPHENATE AMINOTRANSFERASE (PPA-AT [29]) and AROGENATE DEHYDRATASE 1 (ADT1 [30]) that catalyze the formation of Phe were identified, as well as an alternative cytosolic biosynthetic route in which phenylpyruvate aminotransferase uses Tyr as amino donor for the production of Phe [31]. By measuring the emission of volatiles emitted by flowers of transgenic *P. hybrid*a lines, it was discovered that the arogenate pathway is the primary route for the synthesis of Phe, and the cytosolic synthesis of phenylpyruvate was boosted when metabolic input to this pathway is reduced. Similarly, suppressing the flux of Phe from the plastid to the cytosol through silencing of *CATION AMINO ACID PHE TRANSPORTER* (*PhpCAT*) also results in upregulation of the cytosolic Phe biosynthetic route [32]. It is currently unknown whether alternative cellular reservoirs for aromatic amino acid production are present in the cell, and little to nothing is known about Phe catabolism in plants [33]. From the cytosolic pool of Phe, benzenoids ($C_6$–$C_1$), phenylpropanoids ($C_6$–$C_3$ backbone), and phenylpropanoid-related compounds ($C_6$–$C_2$) are synthesized. In a series of reactions that occur in the cytosol, Phe is deaminated by PHENYLALANINE AMMONIA LYASE (PAL) to *trans*-cinnamic acid (CA) and then to benzoic acid (BA), the precursor of benzenoids and phenylpropanoids [34]. Alternatively, in the peroxisomes CA is converted to benzoyl-CoA via the β-oxidative route, and this is then exported to the cytosol where the final steps of the biosynthesis of phenylpropanoids take place [35,36]. The direct conversion of Phe to phenylacetaldehyde has also been reported [37].

Finally, flowers also emit volatile fatty acid derivatives (Figure 2F) – aldehydes, alcohols, and their esters – that are produced in plastids and peroxisomes (Figure 1) from the C18 fatty acids, linoleic and linolenic acid (Figure 2) through the activity of LIPOXYGENASES (LOXs) (Figure 1). Sometimes these compounds are referred to as green leaf volatiles (GLVs) because they are also released when green plant tissue is damaged [38]. Nevertheless, they are also important compounds of the floral bouquet and, for example, mimic the sex pheromones of some insect pollinators [39,40], as well as herbivore-induced volatiles that attract natural enemies of plant herbivores such as caterpillars [41].

Although the pathways leading to the production of volatile compounds are now relatively well established, the question how volatiles are released from cells into the atmosphere is still largely unresolved (Figure 1). In the past, it was suggested to occur via passive diffusion, but it was

recently reasoned that an active biological process must be involved [42]. Evidence for such an active process comes from several studies. For example, in petals of *Petunia*, the expression of an ABC transporter, *PhABCG1*, was shown to be controlled by ODORANT1, a transcription factor that also controls the expression of *5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE* (*EPSPS*), encoding one of the key enzymes in benzenoid biosynthesis [43]. Other evidence for such an active mechanism is the rise in volatiles emission observed when *VESICLE ASSOCIATED MEMBRANE PROTEIN* (*VAMP271*) was downregulated [44]. Given that VAMP271 mediates the trafficking of vesicles to the plasma membrane it was proposed that a mechanism similar to the secretion of hydrophobic compounds (lipids and pigments) may also be involved in the emission of volatiles. Finally, a role for lipid transfer proteins (LTPs) in terpenoid emission, likely in conjunction with an ABC or PDR transporter, has been suggested [45].

## Post-Transcriptional Trade-Off Between Color and Odor

The lack of floral pigmentation that co-occurs with the emission of floral scent is an eye-catching manifestation of the trade-off between color and odor observed in flowers of many species. In some colorless flowers, CAROTENOID CLEAVAGE DIOXYGENASEs (CCDs) contribute to pigment degradation via oxidative cleavage of double bonds redirecting the pathway from the synthesis of colored carotenoids towards the formation of volatile apocarotenoids [46]. The lack of yellow pigmentation in white varieties of *Chrysanthemum morifolium* is due to the activity of a CCD enzyme [47], and this also holds true for the emission of volatile apocarotenoids from *Osmanthus fragrans* [48] and *Crocus sativus* [49,50], which increases as flowers develop and sexually mature. CCD4 enzymes that cleave carotenoids to yield the volatile β-ionone have been also isolated from flowers of *Malus* × *domestica* (apple), *Rosa* × *damascena* (rose), and *Arabidopsis thaliana* [51]. Interestingly, a recent study on the evolution of flower color in the *Brassica*ceae family revealed that a mutation in the functional *CCD4* of the ancestral white-flowered *Brassica oleracea* caused the development of yellow flowers such as those of *B. napus* and *B. carinata*. Because diurnal pollinators generally prefer yellow-colored to white flowers, it has been speculated that the predominance of the yellow-colored lineage of *Brassica* accessions could have arisen from cross-pollination events mediated by the innate preference of pollinators [52].

## Floral Rewards: Nectar and Pollen

Nectar contains sugars, amino acids, and volatile compounds that attract and reward pollinators [53], together with toxins that deter unwanted visitors [54,55]. The chemical composition of nectar varies between plant species, with sucrose, glucose, and fructose constituting between 8% and 80% of its dry weight. Among angiosperms, some nectars are dominated by hexoses and others by sucrose. The hawkmoth- and hummingbird-pollinated self-compatible *Nicotiana attenuata*, for example, produces nectar rich in sucrose and hexoses, as well as numerous secondary metabolites [46]. *Brassica rapa,* which consists of self-compatible and -incompatible varieties, produces hexose-dominated nectar, and *Arabidopsis thaliana*, a self-compatible self-fertilizer, has nectaries that produce volatiles and hexose-rich nectar [56]. A recent study revealed that *SWEET9*, a member of the *SUGARS WILL EVENTUALLY BE EXPORTED* gene family that is located in the plasma membrane, encodes a polypeptide with sucrose transporter activity that is crucial for nectar secretion [56]. Although floral stalk starch is likely mobilized to sucrose and imported into the nectary via the symplasm [57], SWEET9 appears to be responsible for sugar efflux from the nectaries, and an important role has been suggested for sucrose phosphate synthase in the remobilization of nectary starch to support the secretory process [58]. This method of sucrose secretion is likely not confined to floral nectar because species that harbor extrafloral nectaries also contain *SWEET9* homologs. Intriguingly, the latter study additionally demonstrated that SWEET9 was either conserved or has been independently co-opted for nectar secretion in plants of the Rosaceae and

Asteraceae families [56]. Sucrose is delivered to the flowers following the source to sink pathway [58], which includes both SUCROSE UPTAKE TRANSPORTER (SUT) and SWEET transporters. Our understanding of the site of synthesis and supply of precursors for terpenes, phenylpropanoids, and other volatile precursors remains somewhat fragmentary. However, several lines of evidence suggest that these compounds are synthesized in the flower following supply of primary metabolite precursors such as sugars or amino acids produced by photosynthetically active source tissues. First, expression data indicate that the genes encoding constituents of the biosynthetic pathways for the production of Phe and fatty acids from sugars and amino acids are all expressed, and in some instances more abundantly, in floral tissues than in source leaves [59]. Moreover, application of RNA-sequencing technology in model plants has identified biosynthetic genes whose expression is floral- or pollen-specific, proving that, at the very least, these reactions occur exclusively in these tissue types [60]. Second, although there are several inventories of proteins enabling the flow of sugars and amino acids from source to sink, only a few, if any, describe proteins capable of mediating intracellular transport of secondary metabolites. Finally, metabolomics of floral tissues, nectar, and pollen reveals that these tissues have highly distinctive metabolomes – with a considerable number of the constituent metabolites being tissue-specific [61] – a fact that is only consistent with *de novo* biosynthesis *in situ*. Studies of amino acid transporters have revealed the presence of a multitude of transporters with overlapping function, and several of these transporters are highly expressed either in source leaves or floral tissues ([62], recently reviewed in [63]).

## Floral Signals and Pollination Success

Flowers depend on flower-visitors for their reproductive success, and flower-visitors depend on pollen and nectar to nourish themselves and their offspring. Because of this reciprocally beneficial interaction, the evolutionary trajectories of floral signals in unrelated plant lineages have sometimes converged (Box 2 provides a detailed discussion of the evolution of floral signals). Thus, flowers pollinated by animals with similar behavior and morphology share common floral traits, a process referred to as **pollination syndrome**. In respect to color, for example, nocturnally pollinated flowers are often white or pale-yellow, whereas diurnally pollinated flowers display a wide array of chromatic nuances. Because many nocturnal insects have sacrificed color vision to increase contrast sensitivity [64,65], white flowers that glare at night can be found more efficiently. From a biochemical perspective, the loss of color in flowers can be achieved via reduced but also increased production of specific molecules. For example, the white color of the moth-pollinated *Petunia* axillaris flowers is attained by reduced accumulation of anthocyanins, accompanied or not by increased accumulation of flavonoids.

### Box 2. Pollinator-Mediated Evolution of Floral Signals

The diversity of floral signals has evolved under selection mediated by biotic and abiotic ecological factors. Floral signals and their biosynthetic pathways often evolve through co-opting pre-existing pathways and functions. This is evidenced by pathways sharing enzymes such as, for example, the phenylpropanoid and the lignin biosynthetic pathways [115], and a large part of the pathway for pigments such as carotenoids and anthocyanins and floral volatiles is also shared. In **floral mimicry**, a key gene for signal production has evolved by gene duplication from a housekeeping gene involved in fatty acid metabolism [116]. Many volatiles, pigments, and even some rewards are thought to have shifted from a primary defense function to the attraction of pollinators [117,118] as floral signals evolved under the trade-off between attracting mutualists, being cryptic, or even deterring antagonists. This trade-off can be expected to set an ecological limit to the detectability of flowers, and may explain why many flowers are not excessively showy [119]. Some experimental studies have indeed shown that augmentation of floral scent increases herbivory but not necessarily pollination [120]. Thus, **positive directional selection** mediated by pollinators on floral signals may not always lead to an evolutionary increase in signal intensity [121,122]. An elegant way for plants to circumvent such ecological trade-offs is an alteration of floral signals after herbivore attack through **phenotypic plasticity**. As an example of phenotypic plasticity, tomato and cabbage flowers change their floral signaling after herbivore attack as a means of indirect defense [123,124]. As a consequence, the flowers become less attractive to pollinators. Such plasticity is probably adaptive because it limits the negative effects of defense or altered appearance to the times when herbivores are actually attacking a plant.

Because molecules of different chemical origin have different UV spectral properties, the differential accumulation of specific metabolites may lead to changes in pollinator preferences which may not appear obvious to the human eye. In the case of *Petunia axillaris*, reduced accumulation of anthocyanins produces white UV-reflectant flowers, but the flowers are white and UV-absorbent if concomitantly they also accumulate flavonoids. Because nocturnal moths naturally prefer UV-absorbing flowers, accumulation of flavonols leads to higher insect-visitation rates [66,67]. Similar UV spectral traits regulate pollinator preferences for diurnally pollinated flowers. For example, flowers with a UV-absorbing center and UV-reflecting periphery, also called **bull's eye** flowers, are frequently pollinated by bees [68]. This typical UV floral pattern is attained by a discrete distribution of pigments of different biochemical origins: carotenoids in the floral margin and flavonoids in the floral center, which both appear yellow to humans (a discussion on the regulatory genes involved in floral patterning is provided in Box 1). Because studies on insect vision suggest that floral patterns such as the bull's eye and **nectarguides** can only be seen by insects in close proximity to the flowers, these patterns may facilitate insect landing, and signal the direction and describe the attributes of the reward [69–71]. Indeed, a mutation in the *YELLOW UPPER* (*YUP*) gene that in *Mimulus lewisii* underlies carotenoid accumulation in the nectarguides, results in improper orientation of the bees that approach these flowers [72]. From a distance, brightly colored flowers that accumulate large amounts of anthocyanins or carotenoids, or both, may better serve bird pollinators that indeed prefer red-colored or uniformly yellow-colored flowers [68,73,74].

Association between specific floral scents and pollinators has also been described, but primarily for insects because flowers pollinated by birds are usually scentless. For example, the nocturnally moth-pollinated *Petunia axillaris* emits benzenoid volatiles to guide the animals to the flowers [75], and the diurnally pollinated *Mimulus lewisii* emits a blend of d-limonene, β-myrcene, and (*E*)-β-ocimene that attracts bumblebees [76], whereas pollination by scarab beetles is often associated with the emission of methoxylated aromatic volatiles [77]. Recently, it was described that the *de novo* expression of genes for the production of benzenoid compounds drove a shift from bees to moths as pollinators, whereas loss of function of cinnamate-CoA ligase caused a loss of scent that marked the transition from moth to hummingbird pollination [78]. Floral scent is a dynamic trait. The production of volatiles is a function of gene expression, biosynthesis, and sometimes degradation that rapidly change during the day. Furthermore, volatile emission rates increase with temperature and/or light, while the wind propels their dispersal. Thus, floral scent can fulfill far more signaling functions than color, shape, or texture. In addition to serving as distal [54] and proximal [79] attractants of mutualistic pollinators, floral volatiles also repel florivores as well as nectar and pollen thieves, and protect floral organs from bacteria and yeasts. These multiple functions can be attained with the specific dosage of different molecules blended together in one flower bouquet. For example, flowers that emit benzylacetone and nicotine simultaneously attract pollinators and repel nectar thieves [80]. A recently published review uses linalool as an example to describe the multiple physiological and ecological functions that flowers implement with the synthesis of a single volatile molecule [81]. The responses induced by linalool and its derivatives range from attracting pollinators and their herbivore larval offspring to deterring florivores, repelling facultative visitors, and antagonizing bacterial growth; the precise responses can be **enantiomer**-specific. This multitude of functions suggests that linalool has been co-opted to attain novel physiological functionality. From a biochemical perspective, this can be seen as an attempt to gain multifunctionality by channeling available resources in one biosynthetic direction only. Such a strategy becomes crucial to properly allocate resources to reproduction and/or defense if during flowering the plant is being attacked by herbivores and other pests [82]. In addition to linalool, physiological multifunctionality has been described for many other volatiles emitted from flowers [83].

### Box 3. Truthfulness of Floral Signals

In general, floral signals can be 'honest signals' and thus indicate the presence or amount of reward present in a flower. Direct honest signals emanate directly from the reward, such as the scent of nectar and pollen, or humidity [125,126]. Alternatively, floral signals may show a quantitative association with reward despite being emitted from other parts of the flower [127]. A typical example is flower size – because larger flowers may produce more rewards. Sometimes a specific volatile, for example phenylacetaldehyde in *Brassica rapa*, is associated with reward [127]. Because there is no known biochemical connection between phenylacetaldehyde and nectar production, this association has likely evolved through selection by pollinators. Indeed, pollinators may prefer plants with honest signals, because it increases their foraging efficiency, and thus punish 'cheaters' by avoiding them after a visit.

An interesting twist to this story is the fact that clearly not all floral signals are honest. For example, many plants with showy flowers never produce any rewards [128]. Such obligatory rewardlessness is especially common among orchids, but has independently evolved in several other plant families [129]. Until now the molecular basis of rewardlessness, in other words which genes involved in nectar production became non-functional, is unknown. In plant groups such as the orchids, for which nectarlessness has been suggested to be ancestral [130], it would also be interesting to show how nectar production in rewarding lineages evolved at a molecular level. Rewardlessness can be successful when pollination is highly efficient, and the plant does not require many pollinator visits for fertilization of its ovules, and/or plants produce signals that are highly attractive even without an association with reward. Such signals evolve under 'sensory exploitation' – which means that they target a sensory apparatus that has evolved in a different context, for example in the oviposition or mating behavior of the pollinator. The result of this process is sometimes floral mimicry, in other words the flowers imitate a 'model', for example an oviposition substrate or a mating partner. Many aspects of the biochemistry of floral mimicry are yet to be discovered, but mimicry provides some fascinating examples of convergent biochemical evolution, for example in desaturase enzymes in plants whose products mimic insect sex pheromones.

Besides mediating reproductive success, floral signals also act as a filter for pollinator attraction and thus mediate assortative pollinator attraction. The consequence of this can be (partial) reproductive isolation, termed floral isolation [75,84–87]. By contrast, pollinator innate preferences for floral signals can be overridden by **associative learning** that promotes floral constancy, the preferred visitation of flowers of the same type during a foraging bout [88]. Any neutral floral trait (color or scent) for which the animal does not show innate preferences can be associated with a reward (e.g., nectar), typically when the signal is honest [89] (attributes of floral traits are discussed in Box 3). Bumblebees and bees are fast learners, and can pair pollen and nectar with color and odor [70,90], as can ants and other pollinators [83].

## Concluding Remarks

Flower visitation by animal pollinators is driven by the production of floral signals and rewards composed of an array of metabolites that entice the animals. Studied conducted in *Petunia*, *Mimulus*, and *Antirrhinum* established the connecting link between genes underlying the synthesis and regulation of metabolites with flower phenotype and pollinator response [91]. Correlating genotype to phenotype is nowadays facilitated by the availability of larger genetic resources and tools for genetic engineering. However, deciphering animal behavioral responses to floral stimuli is not easy. When nocturnal moths were presented with *Petunia axillaris* plants modified to show conflicting signals (red-colored, volatile-emitting flowers, or white non-emitting flowers), they displayed conflicting behavior and could not make a choice [75]. Experiments with 3D-printed flowers with scent and color added constitute a novel tool to assess animal responses [92]; although for some aspects this approach is successful, the system is limited by the fact that it is static and not dynamic. In addition to shape and texture, floral traits such as volatile emission and nectar secretion change rapidly in response to internal and external stimuli, and, although slower, color also changes (see Outstanding Questions). Assessing animal responses to floral signals in a natural environment, although complicated by the numerous biotic and abiotic variables, still represents the best strategy to have a holistic view of animal responses to floral metabolic signals [54,93,94]. As the phenological mismatch between plants and pollinators increases [95], a better understanding of the genetics and metabolic processes that underpin pollinator preferences could be used to select resilient plants and plants that offer the proper amount of reward for improved ecological

### Outstanding Questions

How can we link genes to metabolic phenotypes and finally to pollinator responses? The search for metabolic differences among species and natural accessions followed by strategies of gene mapping and complementation analysis is the first and necessary step to link phenotype to genotype. Thereafter, experiments that assess the response of animal pollinators to plants that differ for their floral phenotypes will be necessary to establish the link between phenotype and pollinator response. Technological advances in the fields of genomics and metabolomics have facilitated the identification and characterization of the genes that underpin metabolic traits. Furthermore, controlled field experiments with transgenic plants are currently underway to examine pollinator responses to modified floral traits in natural environments.

What are the physiological and ecological costs of floral signal production? Plant exposure to biotic and abiotic stresses during flowering depletes floral resources to pollinators (i.e., production of signals and rewards), and hence impair seed setting and finally plant performance. Studies performed under controlled environment and field experiments that measure plant fitness under adverse conditions can be used to investigate the impact of those stresses on pollination performance.

How can we identify which flower metabolites mediate the interaction between a particular plant species and its pollinator(s)?

plant-pollinator restoration services [96]. The majority of the studies on plant–pollinator interactions have so far been conducted in model plants and in plants of relevance for ecological and evolutionary studies, but far less in plants of agronomical relevance. However, it should be considered that the yield of pollinator-dependent crops is increasing worldwide and, in particular, the yields of crops with high value such as fruits, nuts, and stimulants depend highly on pollination success [97]. Therefore, the introgression of floral metabolic traits in these crops could also be taken into account in plant breeding programs.

## Acknowledgments

M.B. is supported by a Marie Skłodowska-Curie Individual Fellowship (MSCA-IF-2014; grant 656918). The research of F.P.S. is funded by the European Commission 7th Framework Program (FP7/2007-2013, FP7/2007-2011) under grant agreement 281093.

## References

1. Grotewold, E. (2006) The genetics and biochemistry of floral pigments. *Annu Rev Plant Biol* 57, 761–780
2. Sasaki, N. *et al.* (2014) The role of acyl-glucose in anthocyanin modifications. *Molecules* 19, 18747–18766
3. Saito, K. *et al.* (2013) The flavonoid biosynthetic pathway in *Arabidopsis*: structural and genetic diversity. *Plant Physiology and Biochemistry* 72, 21–34
4. Chanoca, A. *et al.* (2015) Anthocyanin vacuolar inclusions form by a microautophagy mechanism. *The Plant Cell* 27, 2545–2559
5. Poustka, F. *et al.* (2007) A trafficking pathway for anthocyanins overlaps with the endoplasmic reticulum-to-vacuole protein-sorting route in *Arabidopsis* and contributes to the formation of vacuolar inclusions. *Plant physiology* 145, 1323–1335
6. Tohge, T. *et al.* (2011) Toward the storage metabolome: profiling the barley vacuole. *Plant physiology* 157, 1469–1482
7. Zhao, D. and Tao, J. (2015) Recent advances on the development and regulation of flower color in ornamental plants. *Frontiers in plant science* 6, 261
8. Fukada-Tanaka, S. *et al.* (2000) Colour-enhancing protein in blue petals. *Nature* 407, 581–581
9. Faraco, M. *et al.* (2014) Hyperacidification of vacuoles by the combined action of two different P-ATPases in the tonoplast determines flower color. *Cell reports* 6, 32–43
10. Vaknin, H. *et al.* (2005) Active anthocyanin degradation in *Brunfelsia calycina* (yesterday-today-tomorrow) flowers. *Planta* 222, 19–26
11. Zipor, G. *et al.* (2015) In planta anthocyanin degradation by a vacuolar class III peroxidase in *Brunfelsia calycina* flowers. *New Phytologist* 205, 653–665
12. Pélabon, C. *et al.* (2015) Blossom colour change after pollination provides carbon for developing seeds. *Functional Ecology* 29, 1137–1143
13. Khan, M.I. and Giridhar, P. (2015) Plant betalains: chemistry and biochemistry. *Phytochemistry* 117, 267–295
14. Brockington, S.F. (2015) Lineage-specific gene radiations underlie the evolution of novel betalain pigmentation in Caryophyllales. *New Phytologist* 207, 1170–1180
15. Tanaka, Y. *et al.* (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal* 54, 733–749
16. Hatlestad, G.J. *et al.* (2012) The beet R locus encodes a new cytochrome P450 required for red betalain production. *Nature genetics* 44, 816–820
17. Hatlestad, G.J. *et al.* (2015) The beet Y locus encodes an anthocyanin MYB-like protein that activates the betalain red pigment pathway. *Nature genetics* 47, 92–96
18. Polturak, G. *et al.* (2016) Elucidation of the first committed step in betalain biosynthesis enables the heterologous engineering of betalain pigments in plants. *New Phytologist* 210, 269–283
19. Suzuki, M. *et al.* (2014) Transposon-mediated mutation of CYP76AD3 affects betalain synthesis and produces variegated flowers in four o'clock (*Mirabilis jalapa*). *Journal of plant physiology* 171, 1586–1590
20. Muhlemann, J.K. *et al.* (2014) Floral volatiles: from biosynthesis to function. *Plant, cell & environment* 37, 1936–1949
21. Degenhardt, J. *et al.* (2009) Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry* 70, 1621–1637
22. Sapir-Mir, M. *et al.* (2008) Peroxisomal localization of *Arabidopsis* isopentenyl diphosphate isomerases suggests that part of the plant isoprenoid mevalonic acid pathway is compartmentalized to peroxisomes. *Plant physiology* 148, 1219–1228
23. Aharoni, A. *et al.* (2005) Volatile science? Metabolic engineering of terpenoids in plants. *Trends in plant science* 10, 594–602
24. Pérez, L.M. *et al.* (1980) Hydrolysis of allylic phosphates by enzymes from the flavedo of *Citrus sinensis*. *Phytochemistry* 19, 183–187
25. Magnard, J.-L. *et al.* (2015) Biosynthesis of monoterpene scent compounds in roses. *Science* 349, 81–83
26. Mintz-Oron, S. *et al.* (2012) Reconstruction of *Arabidopsis* metabolic network models accounting for subcellular compartmentalization and tissue-specificity. *Proceedings of the National Academy of Sciences* 109, 339–344
27. Dong, L. *et al.* (2016) Monoterpene biosynthesis potential of plant subcellular compartments. *New Phytologist* 209, 679–690
28. Maeda, H. and Dudareva, N. (2012) The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annual review of plant biology* 63, 73–105
29. Maeda, H. *et al.* (2011) Prephenate aminotransferase directs plant phenylalanine biosynthesis via arogenate. *Nature chemical biology* 7, 19–21
30. Maeda, H. *et al.* (2010) RNAi suppression of arogenate dehydratase1 reveals that phenylalanine is synthesized predominantly via the arogenate pathway in petunia petals. *The Plant Cell* 22, 832–849
31. Yoo, H. *et al.* (2013) An alternative pathway contributes to phenylalanine biosynthesis in plants via a cytosolic tyrosine: phenylpyruvate aminotransferase. *Nature communications* 4, 2833
32. Widhalm, J.R. *et al.* (2015) Identification of a plastidial phenylalanine exporter that influences flux distribution through the phenylalanine biosynthetic network. *Nature communications* 6, 8142
33. Galili, G. *et al.* (2016) The regulation of essential amino acid synthesis and accumulation in plants. *Annual review of plant biology* 67, 153–178
34. Widhalm, J.R. and Dudareva, N. (2015) A familiar ring to it: biosynthesis of plant benzoic acids. *Molecular plant* 8, 83–97
35. Klempien, A. *et al.* (2012) Contribution of CoA ligases to benzenoid biosynthesis in petunia flowers. *The Plant Cell* 24, 2015–2030
36. Qualley, A.V. *et al.* (2012) Completion of the core β-oxidative pathway of benzoic acid biosynthesis in plants. *Proceedings of the National Academy of Sciences* 109, 16383–16388
37. Tieman, D.M. *et al.* (2007) Tomato phenylacetaldehyde reductases catalyze the last step in the synthesis of the aroma volatile 2-phenylethanol. *Phytochemistry* 68, 2660–2669

38. Matsui, K. (2006) Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Current opinion in plant biology* 9, 274–280
39. Sedeek, K.E. *et al.* (2013) Transcriptome and proteome data reveal candidate genes for pollinator attraction in sexually deceptive orchids. *PLoS One* 8, e64621
40. Schlüter, P.M. *et al.* (2011) Stearoyl-acyl carrier protein desaturases are associated with floral isolation in sexually deceptive orchids. *Proceedings of the National Academy of Sciences* 108, 5696–5701
41. Brodmann, J. *et al.* (2008) Orchids mimic green-leaf volatiles to attract prey-hunting wasps for pollination. *Current Biology* 18, 740–744
42. Widhalm, J.R. *et al.* (2015) Rethinking how volatiles are released from plant cells. *Trends in plant science* 20, 545–550
43. Van Moerkercke, A. (2012) Regulators of floral fragrance production and their target genes in petunia are not exclusively active in the epidermal cells of petals. *Journal of experimental botany* 63, 3157–3171
44. Ting, H.-M. *et al.* (2015) SNARE-RNAi results in higher terpene emission from ectopically expressed caryophyllene synthase in *Nicotiana benthamiana*. *Molecular plant* 8, 454–466
45. Wang, B. *et al.* (2016) Transient production of artemisinin in *Nicotiana benthamiana* is boosted by a specific lipid transfer protein from *A. annua*. *Metabolic engineering* 38, 159–169
46. Hou, X. *et al.* (2016) Synthesis and function of apocarotenoid signals in plants. *Trends in Plant Science* 21, 792–803
47. Ohmiya, A. *et al.* (2006) Carotenoid cleavage dioxygenase (CmCCD4a) contributes to white color formation in chrysanthemum petals. *Plant Physiology* 142, 1193–1201
48. Baldermann, S. *et al.* (2010) Functional characterization of a carotenoid cleavage dioxygenase 1 and its relation to the carotenoid accumulation and volatile emission during the floral development of *Osmanthus fragrans* Lour. *Journal of experimental botany* 61, 2967–2977
49. Frusciante, S. *et al.* (2014) Novel carotenoid cleavage dioxygenase catalyzes the first dedicated step in saffron crocin biosynthesis. *Proceedings of the National Academy of Sciences* 111, 12246–12251
50. Rubio, A. *et al.* (2008) Cytosolic and plastoglobule-targeted carotenoid dioxygenases from *Crocus sativus* are both involved in β-ionone release. *Journal of Biological Chemistry* 283, 24816–24825
51. Huang, F.-C. *et al.* (2009) Cloning and functional characterization of carotenoid cleavage dioxygenase 4 genes. *Journal of experimental botany* 60, 3011–3022
52. Zhang, B. *et al.* (2015) Disruption of a CAROTENOID CLEAVAGE DIOXYGENASE 4 gene converts flower colour from white to yellow in *Brassica* species. *New Phytologist* 206, 1513–1526
53. De la Barrera, E. and Nobel, P.S. (2004) Nectar: properties, floral aspects, and speculations on origin. *Trends in plant science* 9, 65–69
54. Kessler, D. *et al.* (2008) Field experiments with transformed plants reveal the sense of floral scents. *Science* 321, 1200–1202
55. Kessler, D. *et al.* (2012) Unpredictability of nectar nicotine promotes outcrossing by hummingbirds in *Nicotiana attenuata*. *The Plant Journal* 71, 529–538
56. Lin, I.W. *et al.* (2014) Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. *Nature* 508, 546–549
57. Ren, G. *et al.* (2007) Transient starch metabolism in ornamental tobacco floral nectaries regulates nectar composition and release. *Plant Science* 173, 277–290
58. Chen, L.-Q. *et al.* (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 335, 207–211
59. Albert, V.A. *et al.* (2005) Floral gene resources from basal angiosperms for comparative genomics research. *BMC plant biology* 5, 1
60. Kram, B.W. and Carter, C.J. (2009) *Arabidopsis thaliana* as a model for functional nectary analysis. *Sexual plant reproduction* 22, 235–246
61. Yonekura-Sakakibara, K. *et al.* (2014) A flavonoid 3-O-glucoside: 2'-O-glucosyltransferase responsible for terminal modification of pollen-specific flavonols in *Arabidopsis thaliana*. *The Plant Journal* 79, 769–782
62. Hirner, A. *et al.* (2006) Arabidopsis LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. *The Plant Cell* 18, 1931–1946
63. Tegeder, M. (2012) Transporters for amino acids in plant cells: some functions and many unknowns. *Current opinion in plant biology* 15, 315–321
64. Somanathan, H. *et al.* (2009) Resolution and sensitivity of the eyes of the Asian honeybees Apis florea, Apis cerana and Apis dorsata. *Journal of Experimental Biology* 212, 2448–2453
65. Kelber, A. *et al.* (2002) Scotopic colour vision in nocturnal hawkmoths. *Nature* 419, 922–925
66. Sheehan, H. *et al.* (2016) MYB-FL controls gain and loss of floral UV absorbance, a key trait affecting pollinator preference and reproductive isolation. *Nature genetics* 48, 159–166
67. Hoballah, M.E. *et al.* (2007) Single gene-mediated shift in pollinator attraction in *Petunia*. *The Plant Cell* 19, 779–790
68. Papiorek, S. *et al.* (2016) Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns. *Plant Biology* 18, 46–55
69. de Ibarra, N.H. *et al.* (2015) More than colour attraction: behavioural functions of flower patterns. *Current opinion in insect science* 12, 64–70
70. Nicholls, E. and Hempel de Ibarra, N. (2016) Assessment of pollen rewards by foraging bees. In *Functional Ecology*. Published online November 7, 2016. http://dx.doi.org/10.1111/1365-2435.12778
71. Haverkamp, A. *et al.* (2016) Hawkmoths evaluate scenting flowers with the tip of their proboscis. *eLife* 5, e15039
72. Owen, C.R. and Bradshaw, H. (2011) Induced mutations affecting pollinator choice in *Mimulus lewisii* (Phrymaceae). *Arthropod–Plant Interactions* 5, 235–244
73. Rodríguez-Gironés, M.A. and Santamaría, L. (2004) Why are so many bird flowers red? *PLoS Biol* 2, e350
74. Bradshaw, H. and Schemske, D.W. (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* 426, 176–178
75. Klahre, U. *et al.* (2011) Pollinator choice in *Petunia* depends on two major genetic loci for floral scent production. *Current Biology* 21, 730–739
76. Byers, K.J. *et al.* (2014) Floral volatile alleles can contribute to pollinator-mediated reproductive isolation in monkeyflowers (*Mimulus*). *The Plant Journal* 80, 1031–1042
77. Schiestl, F.P. and Dötterl, S. (2012) The evolution of floral scent and olfactory preferences in pollinators: coevolution or preexisting bias? *Evolution* 66, 2042–2055
78. Amrad, A. *et al.* (2016) Gain and loss of floral scent production through changes in structural genes during pollinator-mediated speciation. *Current Biology* 29, 3303–3312
79. Larue, A.A.C. *et al.* (2015) Experimental manipulation of floral scent bouquets restructures flower–visitor interactions in the field. *Journal of Animal Ecology* 85, 396–408
80. Kessler, D. and Baldwin, I.T. (2007) Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. *The Plant Journal* 49, 840–854
81. Raguso, R.A. (2016) More lessons from linalool: insights gained from a ubiquitous floral volatile. *Current Opinion in Plant Biology* 32, 31–36
82. Lucas-Barbosa, D. (2016) Integrating studies on plant–pollinator and plant–herbivore interactions. *Trends in plant science* 21, 125–133
83. Junker, R.R. (2016) Multifunctional and diverse floral scents mediate biotic interactions embedded in communities. In *Deciphering Chemical Language of Plant Communication* (Blande, J. D. and Glinwood, R., eds), pp. 257–282, Springer
84. Grant, V. (1994) Modes and origins of mechanical and ethological isolation in angiosperms. *Proc. Natl. Acad. Sci. USA* 91, 3–10
85. Bischoff, M. *et al.* (2015) Context-dependent reproductive isolation mediated by floral scent and color. *Evolution* 69, 1–13

86. Hodges, S.A. and Arnold, M.L. (1994) Floral and ecological isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proceedings of the National Academy of Science* 91, 2493–2496

87. Waelti, M.O. *et al.* (2008) Floral odour and reproductive isolation in two species of *Silene*. *Journal of Evolutionary Biology* 21, 111–121

88. Brosi, B.J. (2016) Pollinator specialization: from the individual to the community. *New Phytologist* 210, 1190–1194

89. Schiestl, F.P. (2015) Ecology and evolution of floral volatile-mediated information transfer in plants. *New Phytologist* 206, 571–577

90. Katzenberger, T.D. (2013) Salience of multimodal flower cues manipulates initial responses and facilitates learning performance of bumblebees. *Behavioral Ecology and Sociobiology* 67, 1587–1599

91. Yuan, Y.-W. *et al.* (2013) The genetic control of flower–pollinator specificity. *Current opinion in plant biology* 16, 422–428

92. Policha, T. *et al.* (2016) Disentangling visual and olfactory signals in mushroom-mimicking *Dracula* orchids using realistic three-dimensional printed flowers. *New Phytologist* 210, 1058–1071

93. Kessler, D. *et al.* (2013) Petunia flowers solve the defence/apparency dilemma of pollinator attraction by deploying complex floral blends. *Ecology letters* 16, 299–306

94. Pickett, J.A. and Khan, Z.R. (2016) Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytologist* 212, 856–870

95. Kudo, G. and Ida, T.Y. (2013) Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology* 94, 2311–2320

96. Menz, M.H. *et al.* (2011) Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms. *Trends in plant science* 16, 4–12

97. Potts, S.G. *et al.* (2016) *The Assessment Report on Pollinators, Pollination and Food Production: Summary for Policymakers,* Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)

98. Harmer, S.L. (2009) The circadian system in higher plants. *Annual review of plant biology* 60, 357–377

99. Fenske, M.P. *et al.* (2015) Circadian clock gene LATE ELONGATED HYPOCOTYL directly regulates the timing of floral scent emission in *Petunia*. *Proceedings of the National Academy of Sciences* 112, 9775–9780

100. Verdonk, J.C. *et al.* (2005) ODORANT1 regulates fragrance biosynthesis in petunia flowers. *The Plant Cell* 17, 1612–1624

101. Passeri, V. *et al.* (2016) New challenges for the design of high value plant products: stabilization of anthocyanins in plant vacuoles. *Frontiers in plant science* 7, 153

102. Koes, R. *et al.* (2005) Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. *Trends in plant science* 10, 236–242

103. Dubos, C. *et al.* (2010) MYB transcription factors in *Arabidopsis*. *Trends in plant science* 15, 573–581

104. Yuan, Y.-W. *et al.* (2016) Competition between anthocyanin and flavonol biosynthesis produces spatial pattern variation of floral pigments between *Mimulus* species. *Proceedings of the National Academy of Sciences* 113, 2448–2453

105. Whitney, H.M. *et al.* (2013) The influence of pigmentation patterning on bumblebee foraging from flowers of *Antirrhinum majus*. *Naturwissenschaften* 100, 249–256

106. Shang, Y. *et al.* (2011) The molecular basis for venation patterning of pigmentation and its effect on pollinator attraction in flowers of *Antirrhinum*. *New Phytologist* 189, 602–615

107. Albert, N.W. *et al.* (2011) Members of an R2R3-MYB transcription factor family in *Petunia* are developmentally and environmentally regulated to control complex floral and vegetative pigmentation patterning. *The Plant Journal* 65, 771–784

108. Zvi, M.M.B. *et al.* (2008) Interlinking showy traits: co-engineering of scent and colour biosynthesis in flowers. *Plant biotechnology journal* 6, 403–415

109. Zvi, M.M.B. *et al.* (2012) PAP1 transcription factor enhances production of phenylpropanoid and terpenoid scent compounds in rose flowers. *New Phytologist* 195, 335–345

110. Spelt, C. *et al.* (2002) ANTHOCYANIN1 of petunia controls pigment synthesis, vacuolar pH, and seed coat development by genetically distinct mechanisms. *The Plant Cell* 14, 2121–2135

111. Cna'ani, A. *et al.* (2015) Two showy traits, scent emission and pigmentation, are finely coregulated by the MYB transcription factor PH4 in petunia flowers. *New Phytologist* 208, 708–714

112. Butelli, E. *et al.* (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature biotechnology* 26, 1301–1308

113. Tohge, T. *et al.* (2015) Ectopic expression of snapdragon transcription factors facilitates the identification of genes encoding enzymes of anthocyanin decoration in tomato. *The Plant Journal* 83, 686–704

114. Zhang, Y. *et al.* (2015) Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato. *Nature communications* 6, 8635

115. Muhlemann, J.K. *et al.* (2014) The monolignol pathway contributes to the biosynthesis of volatile phenylpropenes in flowers. *New Phytologist* 204, 661–670

116. Schlüter, P.M. *et al.* (2011) Stearoyl-acyl carrier protein desaturases are associated with floral isolation in sexually deceptive orchids. *Proceedings of the National Academy of Sciences of the United States of America* 108, 5696–5701

117. Armbruster, W.S. (1997) Exaptations link evolution of plant–herbivore and plant–pollinator interactions: a phylogenetic inquiry. *Ecology* 78, 1661–1672

118. Schiestl, F.P. (2010) The evolution of floral scent and insect chemical communication. *Ecology Letters* 13, 643–656

119. Shuttleworth, A. and Johnson, S.D. (2012) The *Hemipepsis* wasp-pollination system in South Africa: a comparative analysis of trait convergence in a highly specialized plant guild. *Botanical Journal of the Linnean Society* 168, 278–299

120. Theis, N. and Adler, L.S. (2012) Advertising to the enemy: enhanced floral fragrance increases beetle attraction and reduces plant reproduction. *Ecology* 93, 430–435

121. Gross, K. *et al.* (2016) Why do floral perfumes become different? Region-specific selection on floral scent in a terrestrial orchid. *PloS one* 11, e0147975

122. Parachnowitsch, A.L. (2012) Phenotypic selection to increase floral scent emission, but not flower size or colour in bee-pollinated *Penstemon digitalis*. *New Phytologist* 195, 667–675

123. Schiestl, F.P. *et al.* (2014) Herbivory and floral signaling: phenotypic plasticity and tradeoffs between reproduction and indirect defense. *New Phytologist* 203, 257–266

124. Kessler, A. (2016) Introduction to a virtual special issue on plant volatiles. *New Phytologist* 209, 1333–1337

125. Raguso, R.A. (2004) Why are some floral nectars scented? *Ecology* 85, 1486–1494

126. Von Arx, M. *et al.* (2012) Floral humidity as a reliable sensory cue for profitability assessment by nectar-foraging hawkmoths. *Proceedings of the National Academy of Sciences* 109, 9471–9476

127. Knauer, A. and Schiestl, F. (2015) Bees use honest floral signals as indicators of reward when visiting flowers. *Ecology letters* 18, 135–143

128. Schiestl, F.P. and Johnson, S.D. (2013) Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution* 28, 307–315

129. Renner, S.S. (2006) Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. In *Plant–Pollinator Interactions: From Specialization to Generalization* (Waser, N.M. and Ollerton, J., eds), pp. 123–144, University of Chicago Press

130. Cozzolino, S. and Widmer, A. (2005) Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology & Evolution* 20, 487–494